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EVALUATION OF THE TOXICOKINETIC STUDY OF 1,2-PROPANEDIOL DINITRATE (PGDN) IN THE DOG

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Roger C. Inman

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Chief, Toxic Hazards Division

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Bolus injections of PGDN, an organic dinitrate, were made intravenously to dogs at four (4, 10, 40 and 100 mg/kg) dose levels. Dynamic effects were dose related and both physiological and biochemical in nature. The physiological changes were maximized during the fast distribution phase of PGDN in the central compartment. These included the vasodilation effects of decreased systolic, diastolic and pulse blood pressures. A reflex increase-

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20. ABSTRACT

in heart rate ensued which was significantly dose related with a maximum attainable increase of 129.5 beats per minute.

One biochemical manifestation of PGDN intoxication was an increase in methemoglobin. Values as high as 50% contributed to a delayed drug effect of increased heart rate.

Urine production, presumably changed due to blood pressure, decreased inversely with PGDN dose. An unexpected occurrence associated with this decrease was the appearance of whole blood cells in the urine. Light microscopic histopathology was unable to determine the damage site.

Quantitative techniques, developed for PGDN analysis in blood, urine and expired air, monitored the PGDN elimination processes for approximately five half lives (eight hours). From these data a complex model was developed that described the central compartment elimination equation:

$$C = e^{-0.394t} + e^{-0.054t} + e^{-0.007t}$$

The elimination model is described as a three compartment linear, open system. Microscopic rate constants were determined for the two reversible peripheral compartments ($k_{B,TS}$=0.1283 min^{-1}, $k_{TS,B}$=0.1024 min^{-1}, $k_{B,TD}$=0.9254 min^{-1}, $k_{TD,B}$=0.0142 min^{-1}) and an elimination phase ($k_{B,E}$=0.1158 min^{-1}). The $k_{B,E}$ determined from area under the blood curve calculations was in good agreement with the sums of the rates describing PGDN blood metabolism ($k_{B,M}$=0.0895 min^{-1}, $k_{B,M'}$ = 0.0162 min^{-1}) urine, ($k_{B,U}$=0.0000562 min^{-1} and lung excretion ($k_{B,EA}$=0.000149 min^{-1}) determined separately. Major elimination took place as metabolism in the red blood cell; less than 1% of the dose was eliminated by the kidneys and lungs.

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document was submitted and accepted as the doctoral dissertation of Stanley D. Erk and also constitutes the final report on the intravenous Toxicokinetic Study of 1,2-Propanediol Dinitrate (PGDN) in the dog. The research covered in this report began in May, 1980, was completed in February, 1982 and was performed under Air Force Contract Nos. F33615-76-C-5005 and F33615-80-C-0512. K. C. Back, Chief of the Toxicology Branch was the technical monitor and was succeeded by Ms. M. K. Pinkerton as technical monitor for the Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., served as the Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community & Environmental Medicine. Acknowledgement of appreciation is made to Dr. Charles H. Jarboe for his invaluable guidance and encouragement in completion of the dissertation and to Chris Pfledderer, Capt. John R. Latendresse, II and Ms. Jennifer Scheerschmidt for their support and assistance in the research and preparation of this report.

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CHAPTER I
INTRODUCTION

A. Background. 1,2-Propanediol dinitrate has several synonyms including propylene glycol dinitrate, PGDN and propylene dinitrate all of which have appeared in the literature. PGDN will be the term used throughout this study.

PGDN was originally considered as a replacement for ethylene glycol dinitrate (EGDN) in the manufacture of antifreeze dynamite. This was due to the findings that EGDN could lead to vasodilation, headache, vomiting, shoulder discomfort, anginal syndrome in chronic poisoning and sudden death in acute episodes (Kylin et al., 1966, Sugawara, 1973). Its primary use now is as a fuel component in torpedoes and other weapon systems for the U. S. Navy. PGDN is the major constituent of Otto Fuel II (OFII) to which 22.5% di-n-butyl sebacate, a stabilizer, and 1.5% 2-nitrodiphenylamine, a desensitizer, have been added (JANNAF, 1969, Wyman et al., 1979). The additives appear to be physiologically and biochemically inert (Andersen and Mehl, 1973). Since OFII is resistant to biodegradation and is sparingly water soluble, waste water from torpedo fueling and maintainance shops may present a hazard which can be traced to PGDN toxicity (Kessick et al., 1978).

B. Chemistry. PGDN has the following structure:

$$CH_3\underset{ONO_2}{\underset{|}{C}}HCH_2ONO_2$$

It is a clear liquid having a specific gravity of 1.368 at 20°C and a vapor pressure of 0.09844 mmHg at 25°C (Crater, 1929). When freshly prepared, it is colorless with a pungent odor, but on standing, turns light red-brown (American Conference of Governmental Industrial Hygienists, 1980). PGDN is sparingly soluble in water (20°C) at 0.13 g/100 mL.

The molecular weight of PGDN is 166.09. It has no molecular ion when subjected to electron impact mass spectrometry. The most prominent peak is m/z 46, corresponding to NO_2^+ (Silverstein and Bassler, 1968). Other major peaks that have been mentioned in the literature and confirmed in our laboratory are in Table I.

While the vapor pressure of PGDN in OFII is quite low, conditions exist in which concentrations of 100 to 150 ppm can occur in the workplace.

TABLE I. MAJOR MASS SPECTROMETRIC FRAGMENTS OF PGDN

	Relative Abundance					
m/z	46	30	29	43	90	76
UCI	100	20	15	13	4	4
Frazer & Paul (1968)	100	16	17	12	-	9

C. Toxicology. The studies concluded to date have touched three areas of the toxicology of PGDN. These areas are descriptive, metabolic and behavioral.

Kylin et al., (1966) completed the first work by determining the IP LD_{50} on female albino mice for PGDN as well as EGDN and nitroglycerin (GTN). PGDN and EGDN had similar values, 930 mg/kg and 800 mg/kg respectively, whereas the GTN was more toxic with an LD_{50} of 194 mg/kg. Limited elimination studies of intravenously administered doses were also carried out on rabbits. The results indicated that the initial breakdown was extremely rapid (within minutes). There was no apparent difference in elimination rates among the three tested materials; however, an "unexplained difference" occurred between arterial and venous blood concentrations in each case.

A more thorough lethality study was conducted in the late 1960's by Clark and Litchfield (1969). The LD_{50} was calculated for PGDN on several species and administration routes as listed in Table II. Drug related arterial pressure drop and formation of methemoglobin were correlated to dose. It was found in the rat that maximum blood pressure depression was concurrent with maximum PGDN blood level. Injections of PGDN at its LD_{50} dose caused almost complete conversion of hemoglobin to methemoglobin, indicating methemoglobinemia to be the principal cause of death. Additionally, the appearance and disappearance of metabolites in the blood were followed in vitro and in vivo. The results indicated that PGDN is broken down to inorganic nitrate and nitrite as well as mononitrate esters. Litchfield (1971) further indicated that propylene glycol 2-mononitrate predominates in blood; he speculated a reaction that favored formation of this isomer over propylene glycol 1-mononitrate.

TABLE II. ACUTE LD_{50} OF PGDN*

Species	Sex	Route of Administration	LD_{50} (mg/kg)
Rat	F	P.O.	1190
Rat	F	S.C.	463
Rat	M	S.C.	524
Mouse	F	S.C.	1208
Cat	F	S.C.	200-300

*(Clark and Litchfield, 1969)

Primary skin irritation tests in rabbits conducted by Jones et al., (1972) were negative, while application of PGDN to rabbit eyes produced conjunctival erythema 5 minutes after application. The erythema subsided within 24 hours and the iris and cornea were not involved. PGDN was readily absorbed through the skin.

Rabbits dosed daily with 4 g/kg PGDN via dermal application were weak and cyanotic after the second application; thirteen of 14 animals were dead after the fifth application.

Inhalation studies involving animals were conducted by Jones et al., (1972). Male Sprague Dawley rats were exposed to 65 mg/m^3 (9.57 ppm) PGDN vapors 7 hrs/day, 5 days/wk, for a total of 30 exposures. No toxic effects were seen. Hematologic parameters were unchanged and histopathologic examination of tissue did not show any exposure related lesions. Continuous 90-day inhalation exposures of rats, guinea pigs, monkeys and dogs were conducted at three PGDN concentrations: 67 mg/m^3 (9.87 ppm), 108 mg/m^3 (15.9 ppm) and 236 mg/m^3 (34.76 ppm). Rate of body weight gain was unaffected by PGDN exposure. Decreases in hemoglobin (63%) and hematocrit (37%) were observed in dogs exposed to 236 mg/m^3 PGDN. Methemoglobin levels were elevated in all species with dogs and monkeys showing the most dramatic increase. Iron-positive deposits were present in the livers, spleens and kidneys of dogs and monkeys exposed to 236 mg/m^3 PGDN. Fatty changes in the livers and kidneys were also noted in the animals exposed to PGDN vapors.

Andersen and Mehl (1973) compared the effects of acute doses of PGDN and triethylene glycol dinitrate in rats, mice and guinea pigs. Although both compounds produce methemoglobinemia and hypotension in rats, it was concluded that the toxic responses, e.g. convulsions and tremors, were sufficiently different to indicate different mechanisms of action.

The biochemical effects of PGDN were investigated by Andersen and Smith (1973). Their observations were based on in vitro studies of both human and rat hemolysates and intact red blood cells. The reaction of PGDN was found to be molecular rather than enzymatic and approximately first order. The stoichiometry of the hemolysate was 1.5 hemes oxidized per ester bond broken and 1.9 to 2.3 moles heme oxidized per mole of reacted ester while using whole cells.

Monoamine oxidase (MAO) activity in rabbit livers (in vitro) was examined in light of the assertion that certain MAO inhibitors exert a therapeutic (vasodilation) effect on angina pectoris (Kalin and Kylin, 1969). It was found that PGDN, EGDN and GTN were competitive inhibitors of MAO activity, while their metabolites showed no such effect. The conclusion was drawn that tolerance occurs upon chronic EGDN exposure which, when exposure stops, causes an angina-like effect. In severe cases, vascular spasm may be followed by death when exposure ceases.

Additional metabolic effects of PGDN and similar compounds were noted by Komura (1974). Here, ethanol preference and ethanol metabolism were followed in the rat. Activities of liver alcohol dehydrogenase and acetaldehyde dehydrogenase were inhibited by 21% to 60% by injections of 150 mg/kg of PGDN, EGDN or GTN. The decrease in elimination of both ethanol and acetaldehyde were proportional to the decrease in liver activities in the presence of the dinitrates.

Behavorial testing in conjunction with exposure to PGDN has been reported by several authors. Jones et al., (1972) trained four monkeys to perform a visual discrimination test (VDT). They were then injected with a 4 mg/kg dose, allowed one week of rest and reinjected with a 7 mg/kg dose. Observations while using the VDT at one hour preinjection, immediately after injection and for three one hour periods post injection showed minimal behavorial effects. Another set of monkeys were trained on the VDT or on a visual acuity threshold test (VATT) before being continuously exposed to 262 mg/m^3 (38.5 ppm) for 90 days. Testing each week indicated no changes in avoidance behavior patterns as revealed by the VDT and VATT.

A tentative threshold limit value (TLV) of 0.02 ppm PGDN vapor has been established (American Conference of Governmental Industrial Hygienists, 1981). In 1974, Stewart et al. reported a series of human inhalation exposures to PGDN vapors close to the TLV. Headaches occurred in a majority of the individuals exposed to 0.2 ppm PGDN for 4 hours or more. Repetitive exposure to this

concentration produced tolerance to the headaches. The development of tolerance has previously been described for other organic nitrates. No alteration in blood chemistry values occurred in humans after a single 8 hour exposure or repetitive 8 hour exposures to 0.2 ppm PGDN vapors.

Increasing the concentration of PGDN to 0.5 ppm increased headache severity. After 8 hours of exposure to this concentration, all 3 subjects had abnormal modified Romberg tests. One individual was unable to perform a heel-to-toe test with his eyes open. Blood clinical chemistry values in individuals exposed to 0.5 ppm PGDN were unchanged. Blood nitrate and methemoglobin concentrations also were not elevated above control values. No PGDN was detected in the blood (in vitro) of any subject exposed to any concentration.

Finally, Young et al., (1976) trained primates (Macaca mulatta) on a multiple avoidance schedule including discrete trial cued avoidance and free operant avoidance. They were then exposed to successively increasing concentrations of PGDN from 1.8 mg/m^3 (.26 ppm) to 28.2 mg/m^3 (4.2 ppm) for up to 56 days. While plasma concentrations of PGDN increased each time the chamber concentration increased, there was no measureable change in overall behavior which could have been attributed to general debilitation, sensory deficit or motor dysfunction.

This review represents most, if not all of the published papers on PGDN toxicity. Since most studies correlating PGDN blood values to toxic responses used species with markedly different methemoglobin reductase activity than that of man, a kinetic study involving a species with similar enzyme activity would add greatly to the knowledge of PGDN toxicity.

CHAPTER II
PURPOSE AND SCOPE OF STUDY

The primary aim of this study was to define the kinetics of PGDN elimination after acute intravenous dosing to the dog. Since adequate techniques were not available, methods were developed for PGDN analysis in blood, urine and expired air. From these values, a comprehensive kinetic model was derived.

A secondary objective was to document the dose-response relationship of changes in blood pressure, heart rate and urine production. Methemoglobin formation, another well known result of organic nitrate intoxication, was also measured.

Several dose levels, selected to produce a range of mild to severe toxic effects, were followed out to approximately five half-lives.

Finally, a number of procedures were performed to indicate that the test animals were healthy before and during the experiments. These tests included: a battery of clinical chemistry measurements and hematology analyses; routine P_aCO_2, P_aO_2 and pH measurements; and post mortem examination by a veterinary pathologist.

CHAPTER III
METHODS AND EQUIPMENT

A. Test Material. The PGDN was supplied in the form of "spirits" which was a 10% methanol solution. Concentrated PGDN was obtained by evaporating the methanol from the mixture by passing helium across the liquid surface. The PGDN was washed twice with distilled water to remove final traces of methanol before quality control tests were made. Density values were compared to published data and HPLC analyses were made to detect impurities before the PGDN was used for dosing.

B. Test Species and Environment. Male beagle dogs (Ridglan Farms, Inc.) approximately two years old were used in the study. Dogs were the animal of choice due to convenient size and to human similarity in methemoglobin reductase activity (Stolk and Smith, 1966). The animals were quarantined and examined for disease before use. Dogs unsuitable for other studies, for such reasons as epilepsy, were used in preliminary experiments.

Animals were caged in conformance with the Institute of Laboratory Animal Resource Standards. All cage areas were cleaned daily. Purina Lab Canine Diet #5006 was available until 24 hours predosing. Water, with a maximum hardness of 17 ppm (w/w) measured as calcium carbonates was available ad libitum.

Body weights for dosage purposes were taken 24 hours predose and are tabulated in the appendix. Animal groups for each dose level were formed on the basis of mean body weight and are listed in Table III.

TABLE III. DOSAGE LEVELS, ANIMAL USAGE AND DURATION OF EXPERIMENT

	Dose (mg PGDN/kg)				
	0	4	10	40	100
Animal Numbers and Time on Test	A32-4 hr A96-4 hr A68-8 hr	A72-4 hr A74-4 hr	A90-4 hr A80-8 hr A98-8 hr B20-8 hr B40-8 hr	A88-4 hr A76-8 hr A92-8 hr A94-8 hr B08-8 hr	A86-8 hr
Mean Group Weight(kg)	10.74	12.38	11.54	11.54	12.13

C. Hematology and Clinical Chemistry. Blood samples (22 ml) were taken monthly for a variety of clinical hematology and chemistry tests (Table IV). Calcium measurement was a modification of the calcium o-cresolphthalein complexone complexometric reaction (Anderegg et al., 1954). The albumin analysis method used was an adaptation of the bromcresol green dye binding method of Rodkey (1965). The total protein technique was a modification of the biuret reaction (Kingsley, 1942). The glucose method was an adaptation of the general clinical laboratory method of Stein and Bergmeyer (1963). Alkaline phosphatase was determined from the enzymatic rate measurement of the hydrolysis of p-nitrophenylphosphate (Bessey et al., 1947). The glutamic pyruvic transaminase technique used was an adaptation of the coupled GPT/lactate dihydrogenase procedure (Bergmeyer and Bernt, 1965). The glutamic oxalacetic transaminase method was a modification of the recommended technique of the International Federation of Clinical Chemistry (Saris, 1978). The total bilirubin method was a modification of the classical diazo reaction (van den Bergh and Snapper, 1913). The creatinine determination employed a modification of the Jaffe kinetic reaction (Larsen, 1972). The serum urea nitrogen technique employed a urease/glutamate dehydrogenase coupled enzyme (Talke and Schubert, 1965).

TABLE IV. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON DOGS INFUSED WITH PGDN

Hematology	Chemistry
Hematocrit[a]	Sodium[b]
Hemoglobin[a]	Potassium[b]
RBC[a]	Calcium[c]
WBC[a]	Albumin/Globulin[c]
Differentials	Total Protein[c]
Mean Corpuscular Volume (MCV)[a]	Glucose[c]
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase[c]
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT[c]
	SGOT[c]
	Bilirubin[c]
	BUN[c]

a. Determined on a Hycel HC-500 Counter
b. Determined on an Instrument Laboratory 433 Flamephotometer
c. Determined on a DuPont Automatic Clinical Analyzer

D. Anesthetics and Dosing Techniques. The dog is well suited for use in experiments requiring anesthesia since the central nervous system responds similarly to man (Krantz, Jr. and Cascorbi, 1969). This is also true for extrapolating cardiovascular data for dogs under anesthesia. Since femoral cutdowns were required for these experiments, an anesthestic which has little or no cardiovascular effect was necessary. Two of the more commonly used drugs, morphine and pentobarbital, do not meet this criterion. α-Chloralose appeared to be a good candidate since it has no effect on nerve transmission in autonomic ganglia nor at the myoneural junction (Di Palma, 1965).

Extensive reflex studies have indicated that 4 mg/kg of morphine administered S.C. to beagles (other canines 2 mg/kg) 30 minutes prior to the start of chloralose infusion was appropriate (Chenoweth and Van Dyke, 1969). Since chloralose is only slightly water soluble, it was dissolved in 5% dextrose in lactated Ringers solution (Travenol Laboratories) at a temperature of 55°C (Stecher et al., 1968). To maintain Stage III anesthesia, (Pinniger, 1976) the concentration was kept at 6 mg/ml since a constant infusion into the femoral vein was desired. The infusion rate was adjusted by changing speeds on a Buchler Polystaltic pump.

Dosing, during preliminary studies, was a problem due to PGDN's lack of water solubility. Attempts made to keep PGDN in solution with mixtures of saline and ethanol, saline and acetone, and saline and dimethylsulfoxide were unsuccessful. A surfactant, Tween 80, was found to keep PGDN suspended as numerous small spheres in saline. Since it was later determined that Tween 80 would also cause transitory hypotension in dogs, it could not be used (Newton et al., 1981). Another surfactant, polyethylene glycol 400, (PEG 400) was found to adequately suspend PGDN in saline and not produce hypotension (Budden et al., 1978). The weighed amount of PGDN was added to 8.5 mg PEG 400/kg b.wt. in 10 ml of normal saline and continuously shaken to maintain suspension.

Preliminary studies revealed that the plastic tubing used for infusing both the chloralose and the PGDN would absorb the latter. Therefore, two femoral vein cutdowns were performed. The first was for anesthetic infusion and the second, which only had a short catheter in place, was for PGDN infusion. Since pure PGDN tended to stick in measurable quantities to all plastic surfaces, tubes, caps, 3-way valves and catheters were used once and discarded.

In order to preclude killing the animal due to too rapid dosing, infusion of the 10 ml mixture was carried out over two

minutes. An additional 10 ml of saline was then used to rinse PGDN from the mixing tubes during the following minute. This rinse was then infused over one minute, making a total dose time of four minutes.

E. Blood Gases. Samples of heparinized arterial blood were analyzed periodically for P_aCO_2, P_aO_2 and pH. Results of these tests were used to adjust respiration rate to maintain the dog within acceptable acid-base balance and oxygenation values. A self calibrating (every 1.75 hours) Radiometer Copenhagen Model ABL-2 Acid Base Laboratory was utilized for these determinations. Approximately 1.0 ml of blood was required for each sample. Quality Control buffer samples were made with standard gases on a Dynex tonometer and were analyzed before each experiment.

F. Physiological Responses. All physiological measurements were coordinated through a system designed for this purpose [Hewlett Packard (HP) 7758B and 7758A]. All responses were calibrated at the start of each experiment.

1. Electrocardiogram and Heartrate. The electrocardiogram recorded was from Lead II except for one dog with arrhythmia requiring the aVL lead. These were measured through HP Model 14445A adult disposable electrodes which were placed on shaved and alcohol washed areas of the dog limbs. Signal processing was by a HP Model 8811A unit connected to a HP 8802A Amplifier. The function of all units produced both the ECG and heart rate based on the R wave.

2. Blood Pressure. Continuous arterial blood pressure was directly measured through a Validyne DP 45-14 transducer with a #36 (±258 mmHg) diaphragm placed at heart level. The transducer was connected via polyethylene tubing to a 3-way valve (for blood samples) and to a 16 gauge Jelco teflon catheter. The tubing was flushed every fifteen minutes with heparized saline. The signal was modified by a HP Model 8805D Amplifier for both maximum and minimum pressures.

3. Rectal Temperature. The rectal temperature was measured through a YSI Series 702A Thermilinear probe with thermistor. The signal was processed through a HP 8805C Pressure Amplifier with a temperature bridge adaptor.

If rectal temperatures changed more than several tenths of a degree, an electrical heating pad was applied. Additionally, drapes were placed over the dog to minimize heat loss.

4. Respiration. Dogs were intubated with a 7.0 mm Murphy cuffed endotracheal tube (Harlake #61-02570) that was attached to a Harvard Apparatus Respiration Pump Model #614. The ventilatory volume was maintained at 225 ml; the dual rate control was adjusted to keep P_aO_2 and P_aCO_2 within normal limits.

Measurement of ventilatory rate was through a flow transducer HP 47304A and HP Pneumotach 20072B. The signal from the flow transducer was modified by a HP 8802A amplifier and then integrated by HP 8815A Respiratory Integrator to obtain the minute ventilation.

5. Urine Samples. After application of sterile surgical jelly, a 15 inch premature infant feeding tube (Cutter-Resiflex #14-3901-C11, Size 5 French) was inserted into the animals penis and fed into the bladder for urine collection. The bladder was emptied immediately upon catheterization and at fifteen minute intervals coinciding with the start of dosing. Total volume was noted at each sample time and appropriate amounts retained for analysis. In the event that no urine or less than 1.0 ml was collected, 3.0 ml of saline solution was flushed into the bladder. The saline was then removed if more than 1.0 ml of urine was returned; otherwise, the saline remained in the bladder until the next sampling.

6. Data Acquisition. The physiological measurements were converted to electrical signals for immediate display, long term chart recording and computer processing.

A digital display, Model HP 15050A, sampled the appropriate channels for heart rate, diastolic blood pressure, systolic blood pressure and rectal temperature once each second. The display for each value was updated once every four seconds so that current status of the animal could be observed.

A heat sensitive paper chart recorder (HP 7758A) was used to observe long term trends in rectal temperature, ventilation rate, minute ventilation, heart rate, electrocardiogram, and blood pressure. This recording also was used for non-volatile data storage. Several times problems developed in computer processing resulting in loss of data which were recovered from the chart and entered by hand. Calibration of each channel was also observed on the chart to see if the numbers were rational.

Signals from heart rate, ECG, rectal temperature, arterial blood pressure, diastolic blood pressure, systolic blood pressure, ventilation rate and voice communications were first routed through

a System Controller (HP 5692A) before being directed to an eight channnel Instrumentation Tape Recorder, Model 3968A. This system has the unique advantage in using an all directional microphone to record voice communications. This can be replayed at a later date in case important observaticns were not written down but were talked about. The tape can also be used to re-enter data into the computer if necessary.

The last section of the system contained both data acquisition and data manipulation hardware and software. The controller was a HP 9845B desktop computer interfaced with a HP 7906 Disc Drive, HP 6940B Multiprogrammer, HP 98041A Disc Interface, HP 9878A I/O Expander and HP 9872B Plotter.

Several computer programs that integrated the above equipment specifically for these studies were written. The main features of these programs were: (1) Sample all channels at a rate of once per second and calculate a mean minute value; (2) Display on the CRT a twenty minute time chart of mean heart rate or PGDN concentration with minute blood pressures or P_aO_2 and P_aCO_2 values; and (3) Record and print out in order the calibrated mean minute values of heart rate, diastolic blood pressure, systolic blood pressure and rectal temperature. Other HP programs were used for data transformation and presentation.

G. Methemoglobin Determination. Arterial blood samples (6 µl) were taken for methemoglobin (MetHb) measurement forty five and thiry minutes before dosing began (Rodkey et al., 1979). Samples were thereafter taken 30 minutes apart following the start of dosing. Due to the time required for other procedures, the first of two samples taken was kept at 4°C to prevent further MetHb formation. Tests conducted on blood samples kept at 4°C for several hours and containing both MetHb and PGDN showed no changes in values within the techniques limits.

The procedure called for diluting blood with a carbon monoxide saturated KCN solution producing dual components of COHb-CNMetHb. Spectrophotometric measurements were made at 420 nm before and after addition of sodium hydrosulfite (which converts the solution to a single component COHb system). Calculations from the absorbances produce the fraction of total hemoglobin present as MetHb.

H. PGDN Determination. 1. Mass Spectrometry. One instrument used for PGDN analysis was a Balzers Quadrupole Model QMG511 mass spectrometer with programmable 12 channel selected ion

monitoring and both blood gas catheter and respiratory catheter sampling systems. Instrument conditions are listed in Table V.

TABLE V. MASS SPECTROMETER AND RESPIRATORY CATHETER PARAMETERS

Mass Monitored	90 m/z
Resolution Setting	30
Secondary Electron Multiplier	1800 V
Electrometer	10×10^{-11}
Filter	100 ms
Source Pressure	8×10^{-7} mbar
Emission Current	1 mA
Ionizing Energy	90eV
Catheter Flowrate	13 ml/min
Instrument Response Time	<30 msec
Maximum Breathing Frequency Potential	800 breaths/min

Initial developmental efforts were directed to the use of a teflon or silastic membrane blood gas catheter for PGDN and metabolite analysis. These catheters were designed to be placed within the vein and would permit passive diffusion of the sample vapor into the instrument for analysis while keeping the blood out (Brantigan et al., 1972). Neither membrane catheter proved to be useful even after modification. Both would retain PGDN to such an extent that delays in reaching 90% of the final concentration values were in excess of 20 minutes. The lower limit of detection using the blood gas system was only 1 µg PGDN/ml blood.

The above problems were not true for the respiratory catheter. It was a three meter length of capillary stainless steel tubing sampling expired air at 13 ml/min. Response time was almost immediate. The time to reach 90% of full value was 30 seconds and the lower detection limit of the instrument was less than 1 mg PGDN/m^3 air. This analysis was not available for use until the last dog experiment.

2. Gas Chromatography. Since rapid, continuous blood PGDN analysis was not available by mass spectrometry, other methods were examined. These techniques included colorimetric and gas chromatographic analyses (Litchfield, 1968, Camera and Pravisani, 1967). Most published methods were either insensitive or presented technical problems (peak tailing) that made them unacceptable (Rossel and Bogaert, 1972). Therefore, a gas chromatographic

technique was developed which was rapid, had a lower limit of detection of 10 ng PGDN/ml blood, and covered a recommended three orders of concentration (Erk et al., 1982, Teoreli et al., 1974).

Briefly, the technique used two ether extractions with manual shaking. Sample preparation was complete within 5 minutes. Two microliter aliquots of the samples were analyzed on a HP Model 5880A Gas Chromatograph with a column of 3% base deactivated SP2250 on Supelcoport, a ^{63}Ni electron capture detector and a Level Four Data Integrator. The column was temperature programmed from 70° to 120° at 10°/minute. Examples of typical chromatograms are given in Figure 1. A number of blood samples were spiked with known amounts of PGDN before preparation and analysis. These data were fitted with a second degree polynomial regression model and then plotted (Figure 2). The equation to correct blood analysis for recovery is:

$$y = 0.0664\ x^2 + 0.54795\ x + 0.82919 \qquad (1)$$

The technique was applied to urine samples containing PGDN. Samples were centrifuged for 10 seconds at 3000 RCF (gravities) instead of 30 seconds. Urine samples were spiked with known amounts of PGDN and analyzed. The results are in Table VI. A computer fitted second degree polynomial of

$$y = 0.02336\ x^2 + 0.90471\ x + 0.11968 \qquad (2)$$

was fitted to the logarithm of the data (Figure 3).

I. Euthanasia and Pathology. At the completion of each experiment, the test animal was sacrificed by infusion of 400 mg sodium pentobarbital with concomitant cessation of the respiratory aid.

Necropsy was performed on the four hour test animals the same day of the experiment. The eight hour test animals were necropsied the following day after refrigerated storage. The necropsy consisted of gross examination for abnormalities of all body organs including orifices. Tissues, as listed in Table VII, were sampled, fixed in formalin and retained. Sections of kidney, bladder, prostate and ureter were prepared for histopathology.

Figure 1. Chromatograms of Diethyl Ether Standards and Sample Extract

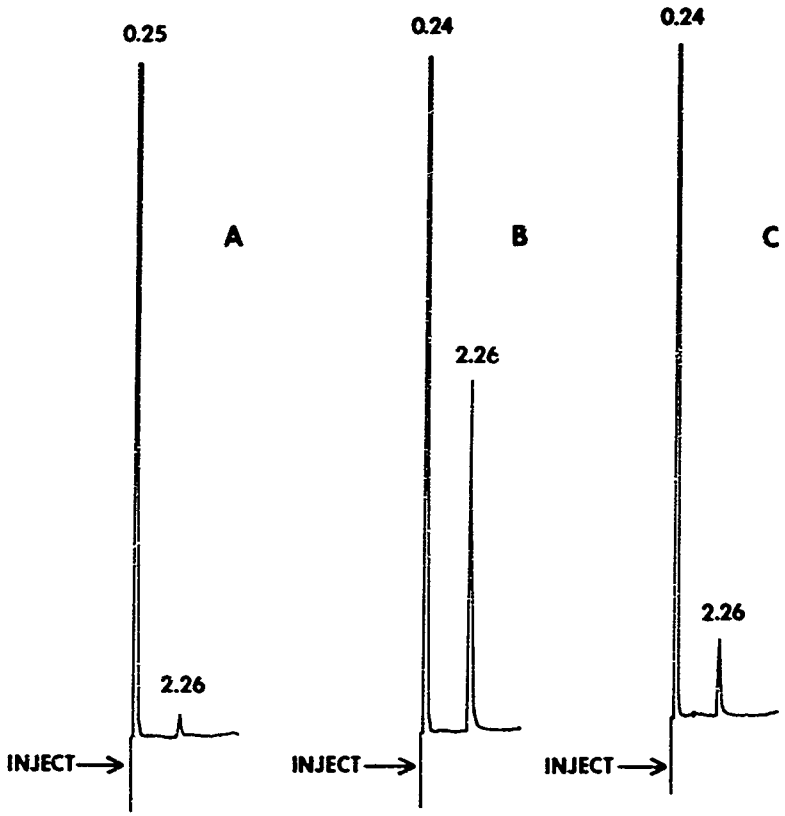


Figure 2. Plot of PGDN Recovery in Blood

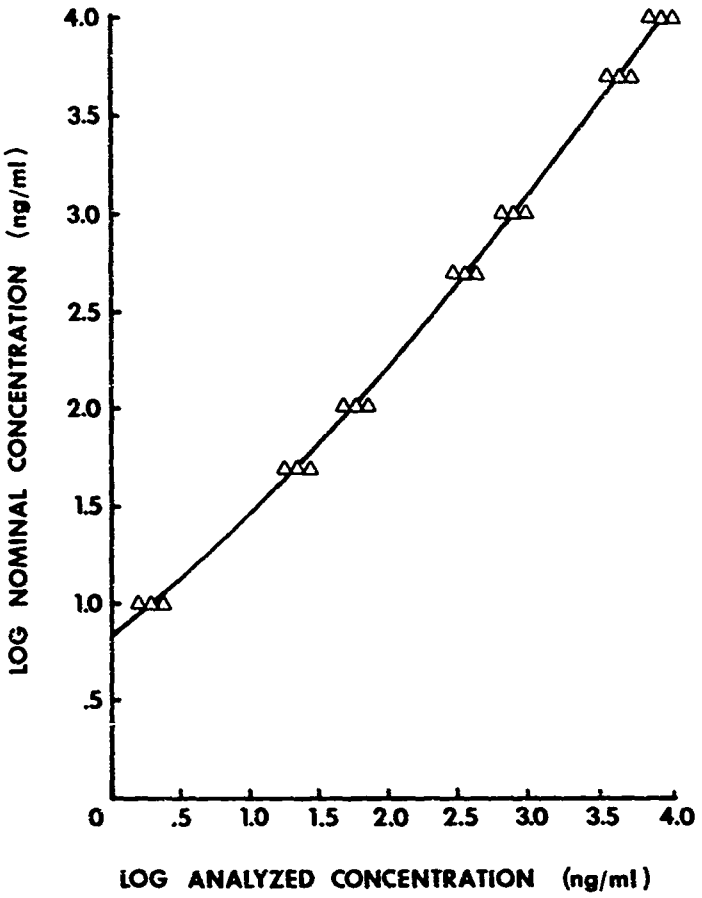


Figure 3. Urine Recovery Data

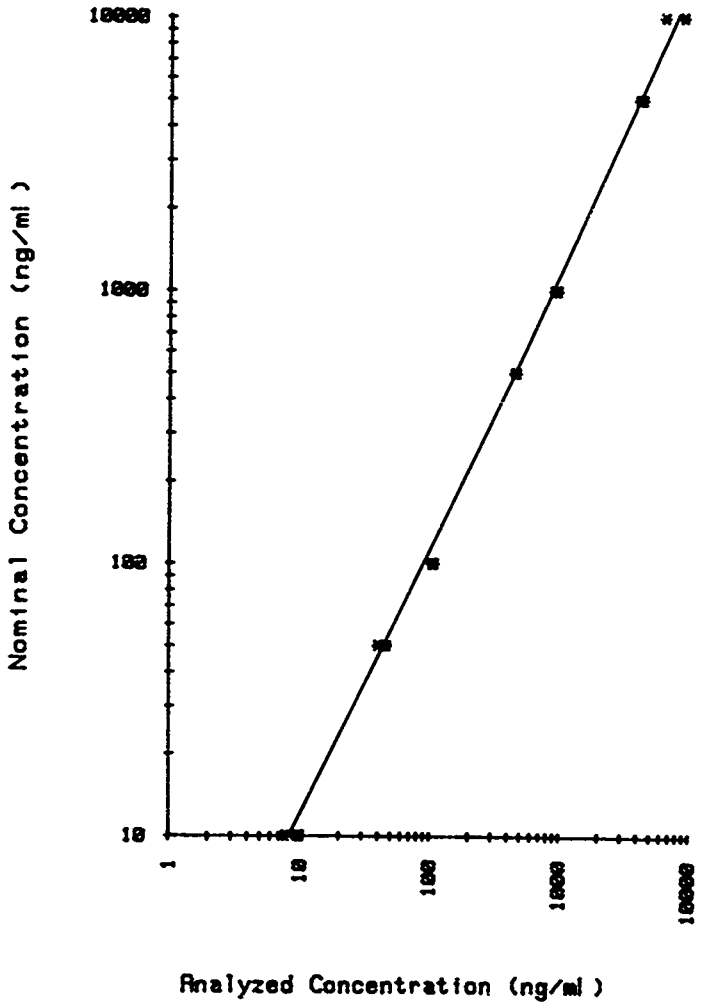


TABLE VI. ACCURACY OF PGDN DETERMINATIONS IN SPIKED URINE SAMPLES

PGDN added (ng/ml)	PGDN found (ng/ml)	% recovery
10000	8795.70	88.0
10000	6263.32	62.6
10000	8744.90	87.4
5000	3995.98	79.9
5000	4267.49	85.3
5000	4094.64	81.9
1000	949.92	95.0
1000	891.27	89.1
1000	921.92	92.2
500	441.72	88.3
500	455.38	91.1
500	458.33	91.7
100	103.01	103.0
100	103.27	103.3
100	106.68	106.7
50	39.34	78.7
50	46.21	92.4
50	44.43	88.9
10	7.78	77.8
10	10.05	100.5
10	9.64	96.4

TABLE VII. TISSUES SAMPLED FROM ANIMALS INFUSED WITH PGDN

Gross Lesions
Tissue Masses of Suspect Tumors and Regional Lymph Nodes
Lungs and Bronchi
Heart
Thyroids
Parathyroid
Mesenteric Lymph Nodes
Thymus
Gall Bladder
Pancreas
Adrenals
Stomach
Pituitary
Duodenum
Ileum
Colon
Liver
Spleen
Kidneys
Bladder
Brain
Prostate
Ureter

CHAPTER IV
RESULTS

A. Blood and Clinical Chemistry. Routine hematology and clinical chemistry determinations were performed monthly to assess overall animal health. The test values were compared with baseline data for nearly 900 dogs. With few exceptions the dogs were within these limits (Table VIII) and judged usable.

TABLE VIII. PREDOSE CLINICAL CHEMISTRY AND HEMATOLOGY RESULTS

Dog Number	A88	A86	A96	A72	A90
Expt. Date	9 Nov	11 Nov	13 Nov	16 Nov	19 Nov
Dose (mg/kg)	40	100	0	4	10
RBC+ 10^6/mm	7.31	6.66	7.01	8.09	7.08
WBC+ 10^3/mm	10.0	13.9	12.4	9.3	10.3
HCT+ %	50	43	46	55	48
HGB+ g/dl	17.4	15.2	16.2	18.7	16.6
MCV+ μm^3	68.4	64.6	65.6	68.0	67.8
MCH+ pg	23.8	22.8	23.1	23.1	23.5
MCHC+ g/dl	34.8	35.4	35.2	34.0	34.6
Bands %	--	2	4	3	7
Neutrophils %	65	84	50	59	59
Lymphocytes %	33	11	36	29	25
Monocytes %	1	--	5	3	7
Eosinophils %	1	3	5	6	2
Sodium mEq/l	157	157	152	155	154
Potassium mEq/l	5.1	5.1	4.9	5.2	5.0
Calcium mg/dl	10.3	10.2	10.6	10.2	10.6
Glucose mg/dl	98	103	125	98	91
T Protein+ g/dl	6.8	7.9*	6.9	7.0	7.2
Albumin g/dl	3.2	3.4	3.5	3.4	3.3
Globulin g/dl	3.6	4.5*	3.4	3.6	3.9*
SGOT+ IU/l	24	32	33	30	27
SGPT+ IU/l	30	40	40	48	42
Alk Phos+ IU/l	3.8	4.0	5.7	2.0	5.8
Bilirubin mg/dl	0.30	0.30	0.24	0.34	0.24
BUN+ mg/dl	11.0	16.2	13.0	15.4	13.8
Creatinine mg/dl	0.9	0.9	0.9	0.9	0.8

TABLE VIII. (CONTINUED)

Dog Number	A74	A94	B20	A68	B08
Expt. Date	24 Nov	30 Nov	2 Dec	4 Dec	7 Dec
Dose (mg/kg)	4	40	10	0	40
RBC+ 10^6/mm	8.94*	9.08*	7.26	9.04*	8.56
WBC+ 10^3/mm	9.1	9.1	8.5	9.9	8.9
HCT+ %	51	56	50	53	53
HGB+ g/dl	18.1	18.1	17.4	18.5	18.0
MCV+ μm^3	57.1	61.7	68.9	58.6	61.9
MCH+ pg	20.3	19.8	24.0	20.5	21.0
MCHC+ g/dl	355	32.3	34.8	34.9	34.0
Bands %	1	1	2	1	3
Neutrophils %	63	60	56	74	45
Lymphocytes %	35	27	38	20	42
Monocytes %	--	4	2	1	2
Eosinophils %	1	8	4	5	8
Sodium mEq/l	147	151	--	149	153
Potassium mEq/l	4.7	4.6	--	5.5	4.6
Calcium mg/dl	10.1	10.3	9.6	9.3*	10.2
Glucose mg/dl	102	106	132	112	111
T Protein+ g/dl	7.0	7.1	6.9	7.5*	6.9
Albumin g/dl	3.3	3.4	3.8	3.6	3.5
Globulin g/dl	3.7	3.7	3.1	3.9*	3.4
SGOT+ IU/l	26	28	49	28	20
SGPT+ IU/l	34	46	50	48	36
Alk Phos+ IU/l	3.0	3.4	4.0	5.1	1.6
Bilirubin mg/dl	0.50	0.40	--	0.50	0.40
BUN+ mg/dl	13.0	8.8	--	9.0	7.0
Creatinine mg/dl	0.9	0.7	--	0.7	0.6

TABLE VIII. (CONTINUED)

Dog Number	A92	A34	B40	A80	A98	A76
Expt. Date	9 Dec	11 Dec	14 Dec	16 Dec	4 Jan	6 Jan
Dose (mg/kg)	40	0	10	10	10	40
RBC+ 10^5/mm	8.92*	7.54	7.59	8.62	9.97*	8.43
WBC+ 10^3/mm	8.1	7.9	7.9	10.1	9.6	8.0
HCT+ %	51	52	46	51	52	48
HGB+ g/dl	18.2	18.0	16.6	18.8	18.4	17.8
MCV+ μm^3	57.2	69.0	60.6	59.2	52.2*	56.9
MCH+ pg	20.4	23.9	21.9	21.8	18.5*	21.1
MCHC+ g/dl	35.7	34.6	36.1	36.9	35.4	37.1
Bands %	1	--	3	1	2	4
Neutrophils %	62	51	54	65	69	50
Lymphocytes %	32	34	29	22	25	33
Monocytes %	--	--	4	1	2	2
Eosinophils %	5	12	10	11	2	11
Sodium mEq/l	143	153	155	155	155	157
Potassium mEq/l	5.2	5.3	4.4	4.9	4.7	4.4
Calcium mg/dl	9.9	9.7	9.8	10.1	10.3	10.5
Glucose mg/dl	99	99	108	114	113	111
T Protein+ g/dl	7.7*	7.3	6.1	7.2	7.1	7.2
Albumin g/dl	3.3	3.3	3.1	3.5	3.6	3.5
Globulin g/dl	4.4*	4.0*	3.0	3.7	3.5	3.7
SGOT IU/l	28	31	25	19	26	27
SGPT+ IU/l	100*	30	44	44	64	30
Alk Phos+ IU/l	3.6	2.5	1.5*	6.1	4.7	1.8
Bilirubin mg/dl	0.40	0.50	0.30	0.40	0.40	0.50
BUN+ mg/dl	10.2	11.6	9.4	12.6	11.0	10.2
Creatinine mg/dl	0.8	0.9	0.5	0.8	0.6	0.8

* Outside 99% Confidence limits of baseline dogs.

+ RBC = red blood cell; WBC = white blood cell; HCT = hematocrit; HGB = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; SGOT = serum glutamic oxalacetic transaminase; SGPT = serum glutamic pyruvic transaminase; Alk Phos = alkaline phosphatase; BUN = serum urea nitrogen; T. Protein = Total Protein.

B. Blood Gases and Rectal Temperatures. Blood gases and rectal temperatures were measured throughout the experiments so that respiration rate and body heat could be adjusted within normal limits. The individual values for each dog are listed in the Appendix. The range of blood gases (Table IX) show that pH was

consistently low while P_aO_2 and P_aCO_2 values were grouped around the ideals of 100 and 40 mmHg, respectively. It is probable that chloralose anesthetic caused a non-respiratory acidemia as reported by Ledsome et al. (1971).

TABLE IX. RANGE OF P_aO_2, P_aCO_2 and pH VALUES DURING EXPERIMENTATION

Dog#	Dose (mg/kg)	P_aO_2 (mmHg)	P_aCO_2 (mmHg)	pH
A34	0	94-107	35-44	7.26-7.38
A68	0	96-109	38-44	7.33-7.39
A96	0	87-107	35-46	7.27-7.35
A72	4	96-105	36-41	7.30-7.35
A74	4	100-106	39-43	7.26-7.30
A80	10	92-109	38-46	7.28-7.39
A90	10	96-102	38-43	7.21-7.29
A98	10	96-105	37-45	7.26-7.33
B20	10	89-98	37-43	7.29-7.33
B40	10	95-128	36-44	7.31-7.39
A76	40	92-97	39-43	7.34-7.38
A88	40	96-122	32-41	7.25-7.37
A92	40	92-111	38-44	7.29-7.36
A94	40	90-100	37-44	7.25-7.33
B08	40	94-103	34-42	7.26-7.33
A86	100	87-100	37-46	7.22-7.31
All Animals		87-128	32-46	7.21-7.39

The range of rectal temperatures was from 35.7°C to 39°C except for the highest dose animal, A86, whose peak temperature was 42°C. We believe the toxic effect of the 100 mg/kg PGDN dose contributed greatly to this finding. The mean rectal temperature of all dogs was 37.5°C.

C. Physiological Responses 1. Heart Rate. The pre (30 minutes) and post dosing heart rates for each dog are in the appendix. To spot general trends the mean minute observation was calculated for each group according to dose level. The mean values are in Figures 4 - 7. Each graph includes control data for comparison. Abrupt changes periodically occur in the plots (e.g. Figure 4, time 220 to 238 minutes; Figure 5 at 240 minutes; and Figure 6 at 45, 120, 150, 165, 210, 240 and 330 minutes). These are artifacts induced into the mean value by changing the N number during calculation. This change in N value is due to either deleting erroneous data due to equipment malfunction or to extending experiments past the original four hours to eight hours.

Figure 4. Mean Heart Rate @ 0 & 4 mg/kg

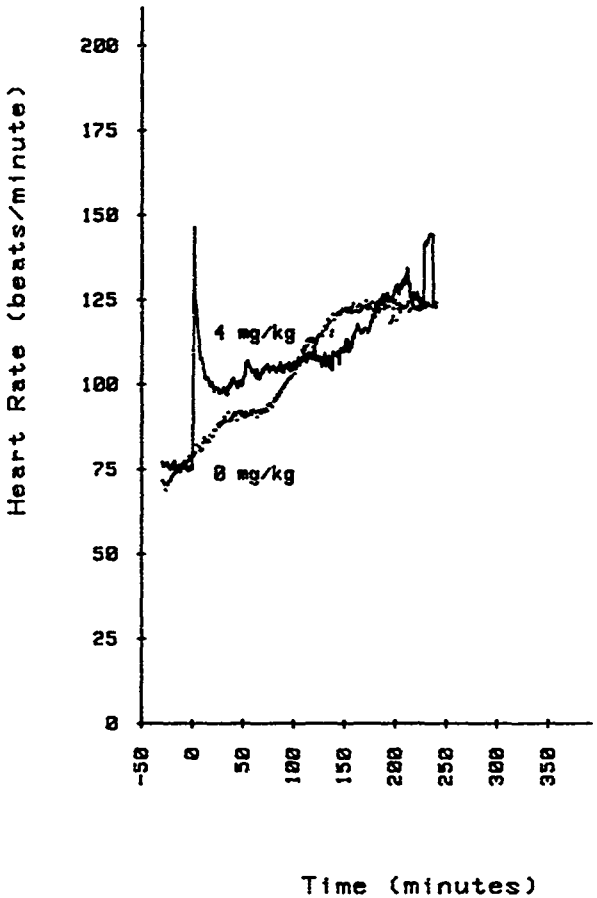


Figure 5. Mean Heart Rate @ 0 & 10 mg/kg

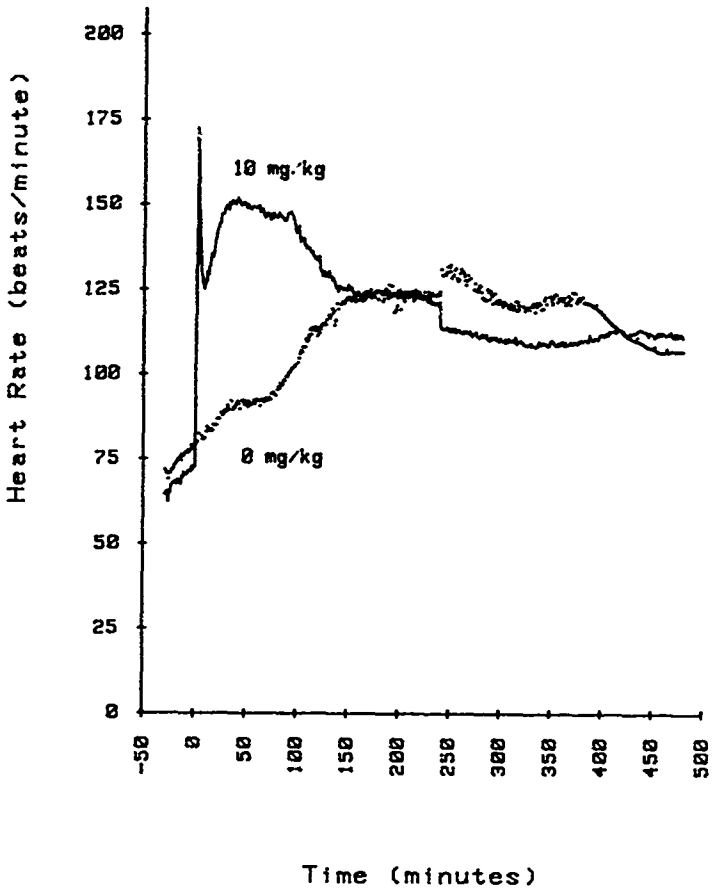


Figure 6. Mean Heart Rate @ 0 & 40 mg/kg

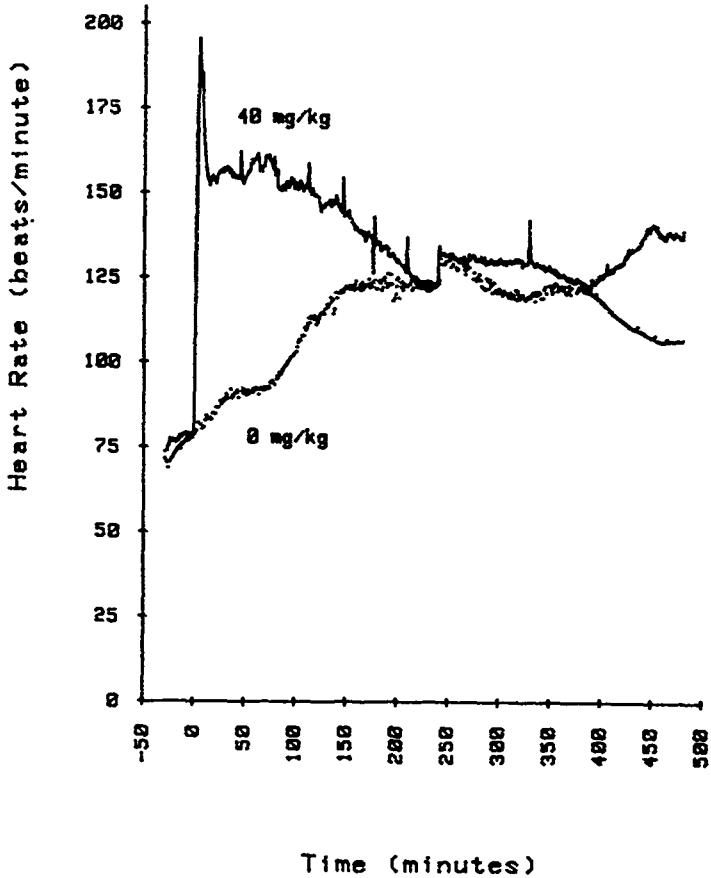
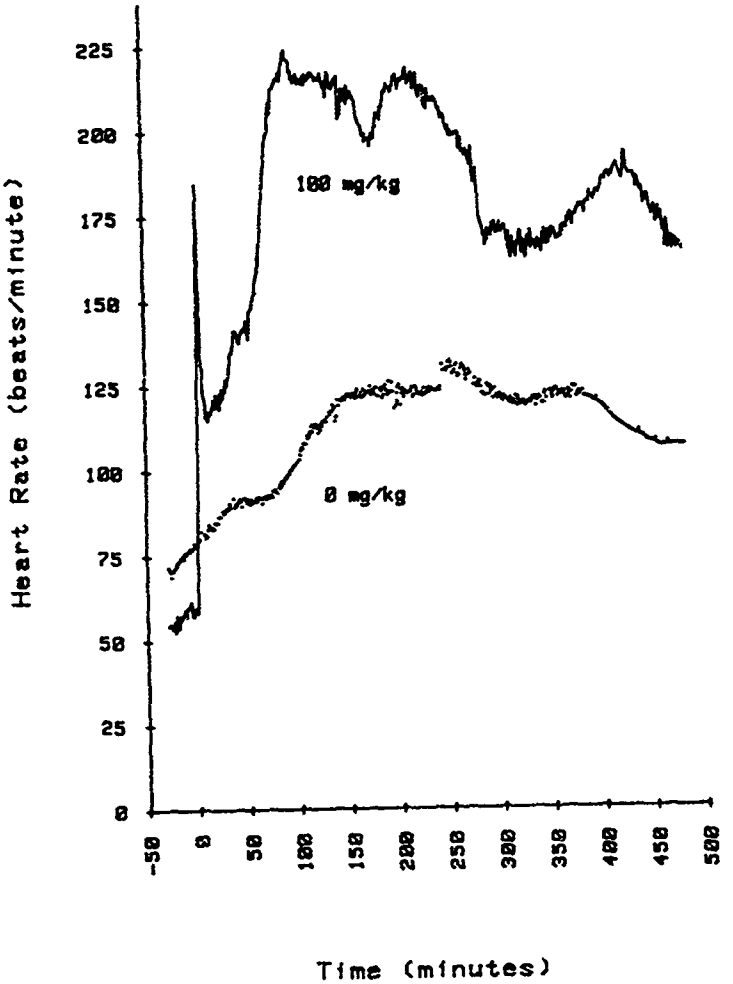


Figure 7. Mean Heart Rate @ 0 & 100 mg/kg



The plots show that the increase in heart rate is PGDN dose related. The largest part of the effect is over within several minutes. Examination of the minute by minute data indicates that the spike occurs during the four minute dosing period. In all cases except the 100 mg/kg dose the heart rate decreases after the initial increase until it closely matches that of the controls. In connection with the 100 mg/kg dose, Kiese (1974) has indicated that MetHb values (discussed in a later section) above 40% will increase heart rate. The MetHb for dog A86 increases to above 40% as the heart rate dramatically increases (at about 90 minutes) and may be a leading cause for this difference.

An unexpected observation was the gradual increase in control dog heart rate. It was presumed from the earlier references that α-chloralose would not affect heart rate. Further investigation into α-choloralose use indicated that the level of anesthesia was to be maintained at an even and light depth, i.e. without spontaneous movements or response to painful stimuli, but with increased tendon reflexes and reactions to sound stimuli (Koppermann et al., 1955). Arfors (1970) and Mauck (1965) found that heart rate would increase, particularly over a long term experiment (6 hours or longer), if there was deep anesthesia as was the case in this study. Taking this into consideration, it was to be expected that the heart rate would gradually increase.

Since α-chloralose effects can be assumed to be similar during all doses, heart rate differences between control and PGDN dosed can be indirectly ascribed to PGDN, its metabolites and methemoglobin formation. A graph of this relationship in terms of time is shown in Figure 8. The 100 mg/kg dose was not included since it never returned to control levels due to MetHb (see above). The figure illustrates that the duration of long term heart rate effects are linearly related to the logarithm of the dose. This indicates the mechanisms that respond to PGDN and its metabolites (that are cardioactive) have not been saturated. Examples of this type relationship have long been recognized (Maher et al., 1962).

Further correlations of the data during dosing and immediately predose are on Figures 9, 10 and 11. Figure 9 is a bar graph which presents mean heart rates, the standard error of the mean and the significant differences between the two correlated sample means at each dose level. Significance was calculated from the t test (Ferguson, 1966):

$$t = \frac{\Sigma D}{[N\Sigma D^2 - (\Sigma D)^2]/(N-1)} \qquad (3)$$

Figure 8. Duration of Increased Heart Rate

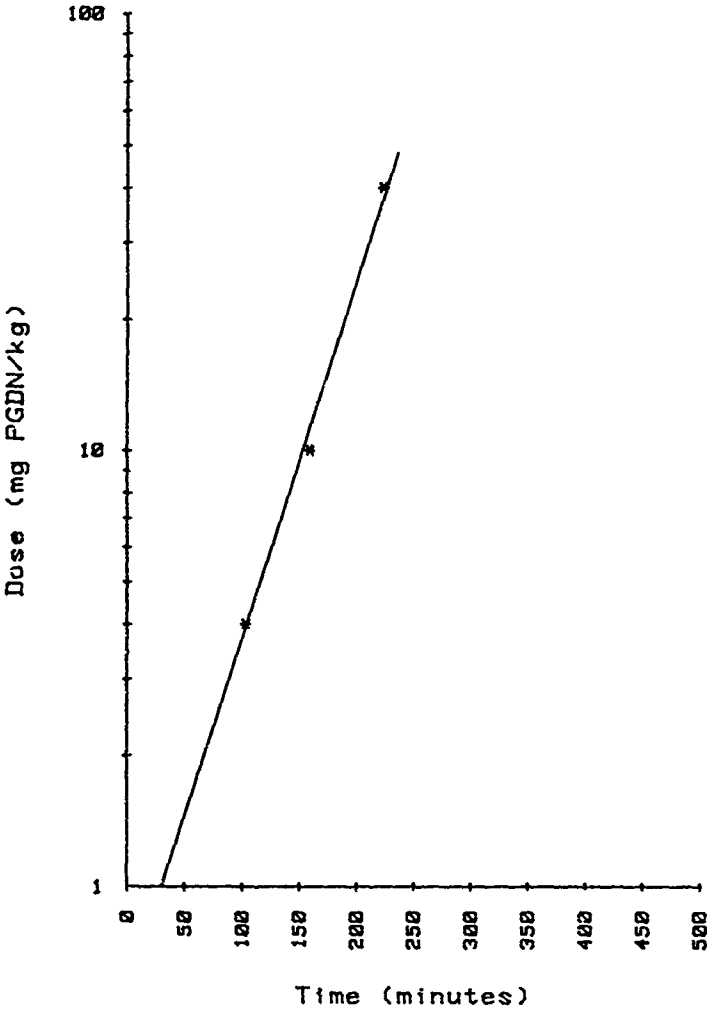


Figure 9. Predose Heart Rate & Maximum Heart Rate During Dosing

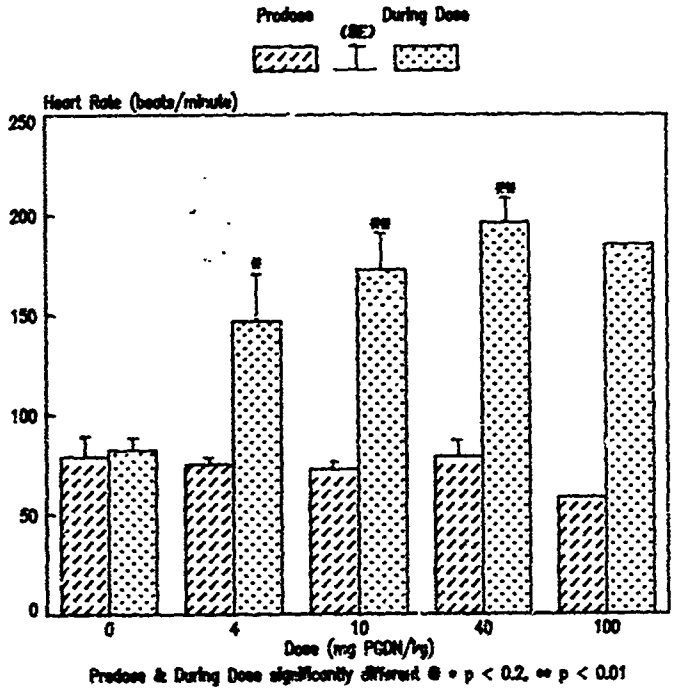


Figure 10. Maximum Increase in Mean Heart Rate During Dosing

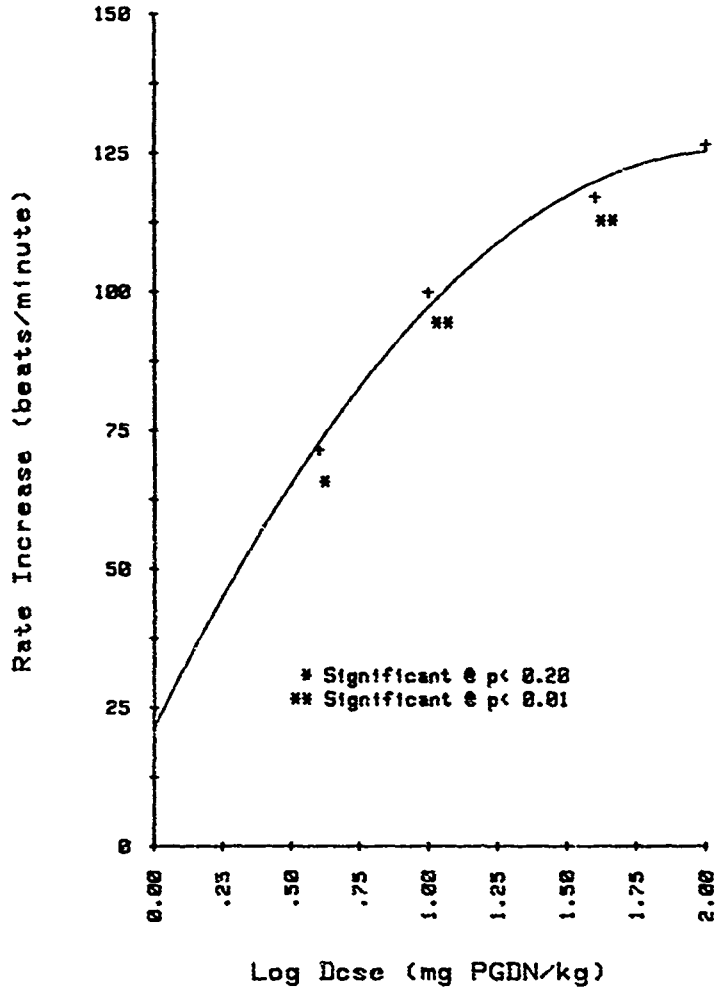
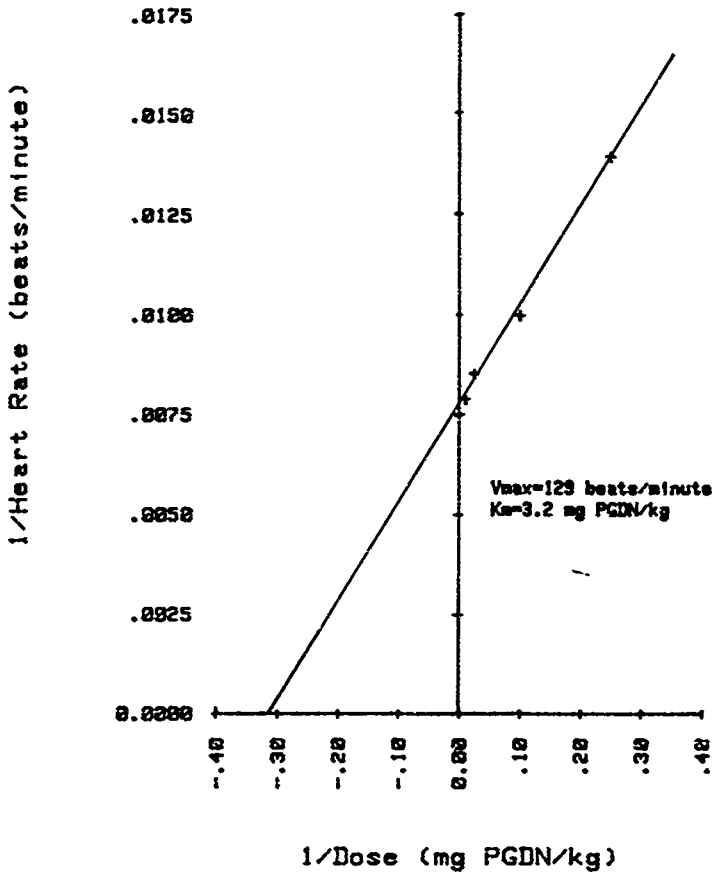


Figure 11. Maximum Heart Rate Increase During Dosing



The increases in heart rate over predose levels were calculated and plotted (Figure 10). The curve of the plot suggests that the heart may be beating close to maximum rate. Since this is similar to a saturable mechanism, a Lineweaver-Burk transformation of the data would allow linear regression techniques to be applied (Figure 11). Chen and Russell (1950) applied this technique to their classic study of cardiovascular drugs in dogs with good results. From the regression analysis of the line, the maximum heart increase during dosing would be 129.5 beats per minute. The Km or dose where one half of the maximum heart rate increase occurs was calculated to be 3.2 mg/kg which was just below the lowest dose given in the study.

2. Systolic Blood Pressure. The pre (30 minutes) and post dosing systolic blood pressures are graphed for each dog in the appendix. As with the heart rate data, mean minute observations were calculated and plotted for each dose level on Figures 12 through 15. The deletion of false data due to blood sampling caused considerable fluctuation in the mean values, particularly in the 4 mg/kg level where only two animals were used. Much more variation was also noted in the individual measurements throughout the experiments. For this reason further data analysis was limited to predose and during dose observations. Figure 16 illustrates the maximum decrease in systolic pressure due to PGDN. The only levels where the drop was significant ($P<0.001$) were the 10 and 40 mg/kg doses. The large standard error for the 4 mg/kg dose may have been the reason for not finding a significant difference between pre and post dose values.

3. Diastolic Blood Pressure. The pre(30 minute) and post diastolic treatment blood pressures graphs are presented for each dog in the appendix. As with systolic pressures, the mean of each minute observation was calculated and plotted for each dose level in Figures 17 to 20. As with the other plots of this type, some fluctuation in values was due to drawing arterial blood samples from the catheter which was also connected to a pressure transducer.

Overall diastolic pressures were much more stable than systolic. The decrease in pressure due to the PGDN dose is readily apparent at all but the highest dose. The pre and during dose values are shown in Figure 21. Three of the five levels have significant differences between the pre and during dose measurements. Of the two remaining, the 100 mg/kg level cannot be tested since it was tried in only one animal. The control (0 mg/kg) values do not show a significant difference.

Figure 12. Mean Systolic Pressure @ 0 & 4 mg/kg

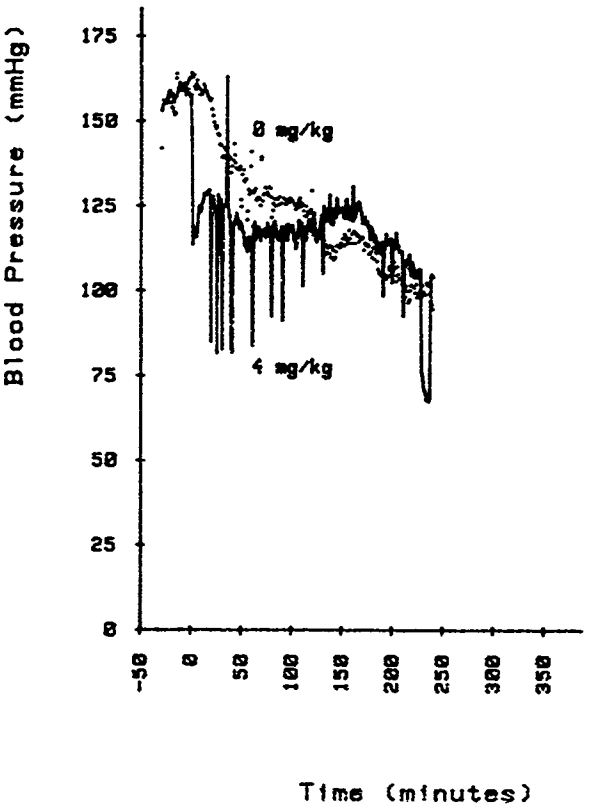


Figure 13. Mean Systolic Pressure @ 0 & 10 mg/kg

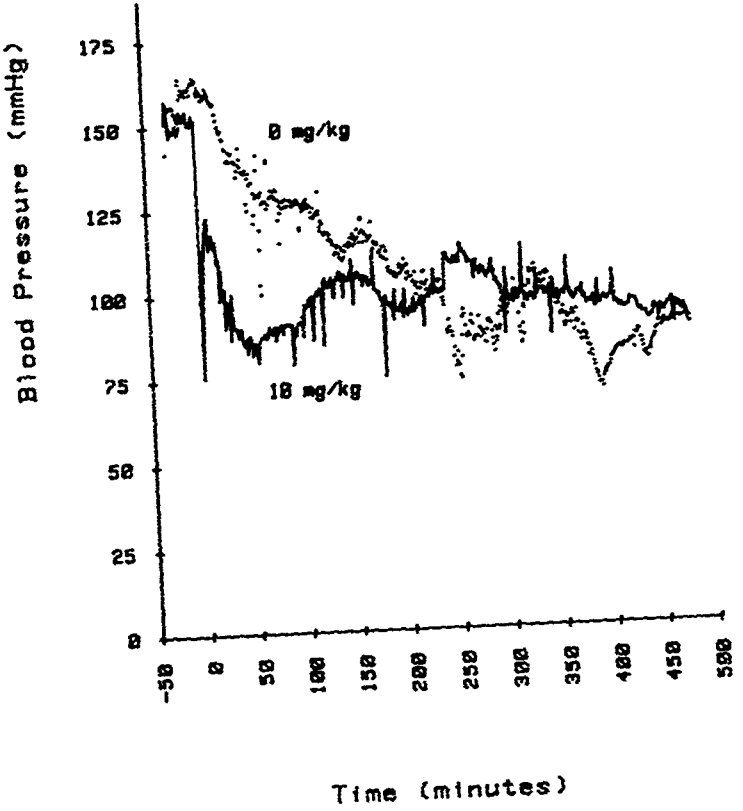


Figure 14. Mean Systolic Pressure @ 0 & 40 mg/kg

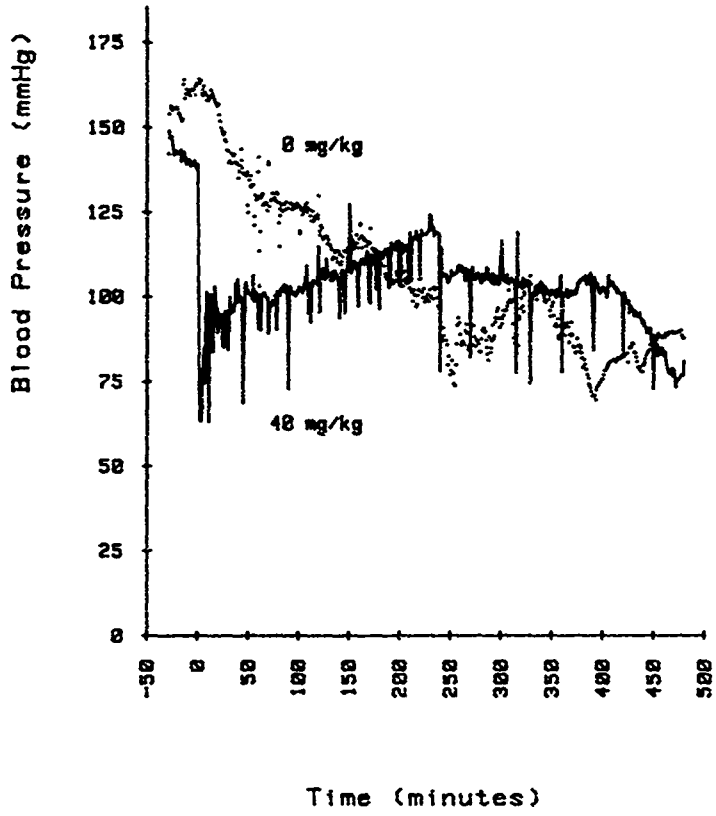


Figure 15. Mean Systolic Pressure @ 0 & 100 mg/kg

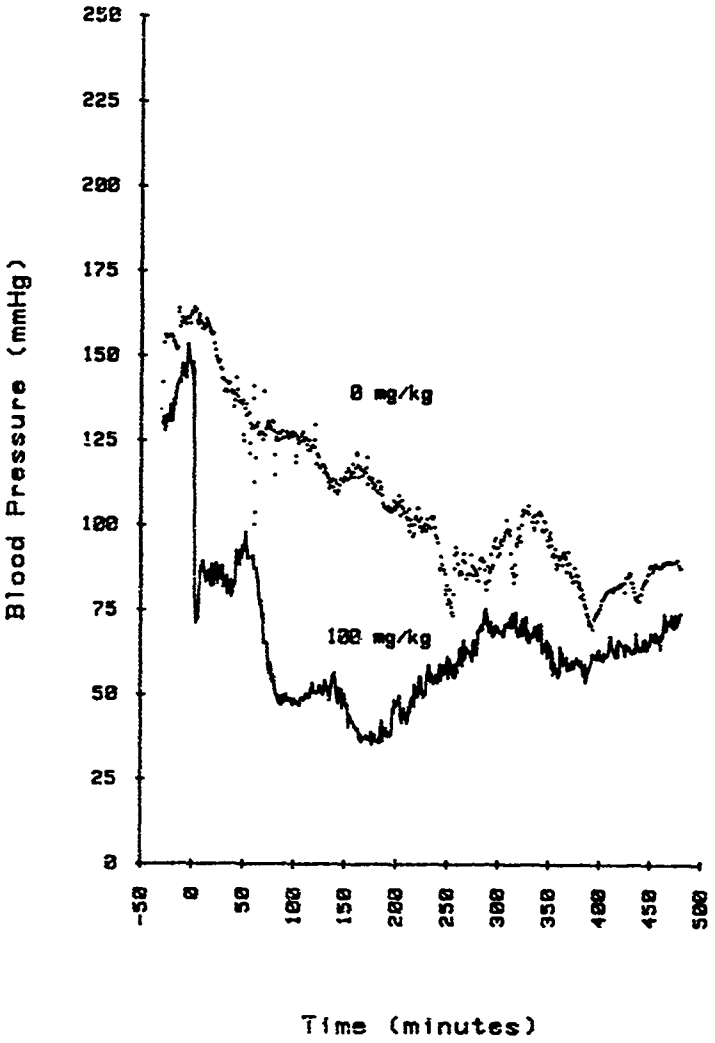


Figure 16. Predose Systolic Blood Pressure & Minimum Systolic Blood Pressure During Dosing

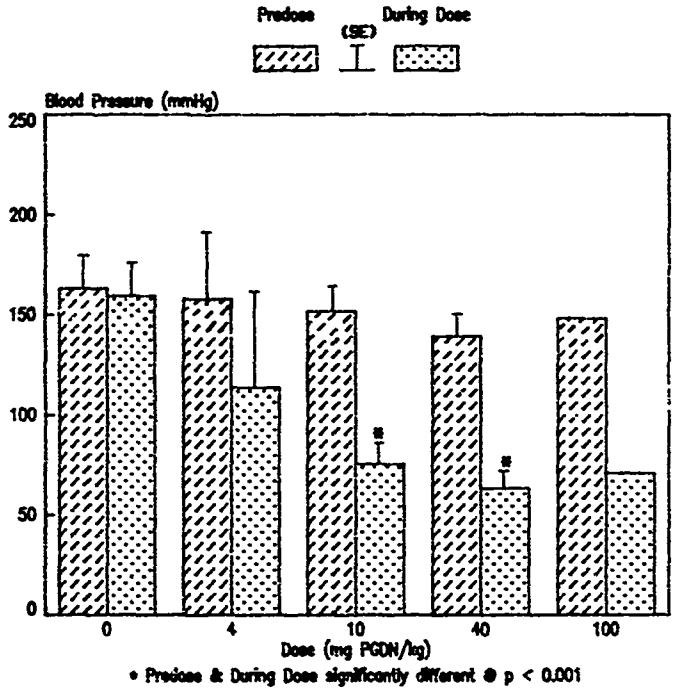


Figure 17. Mean Diastolic Pressure @ 0 & 4 mg/kg

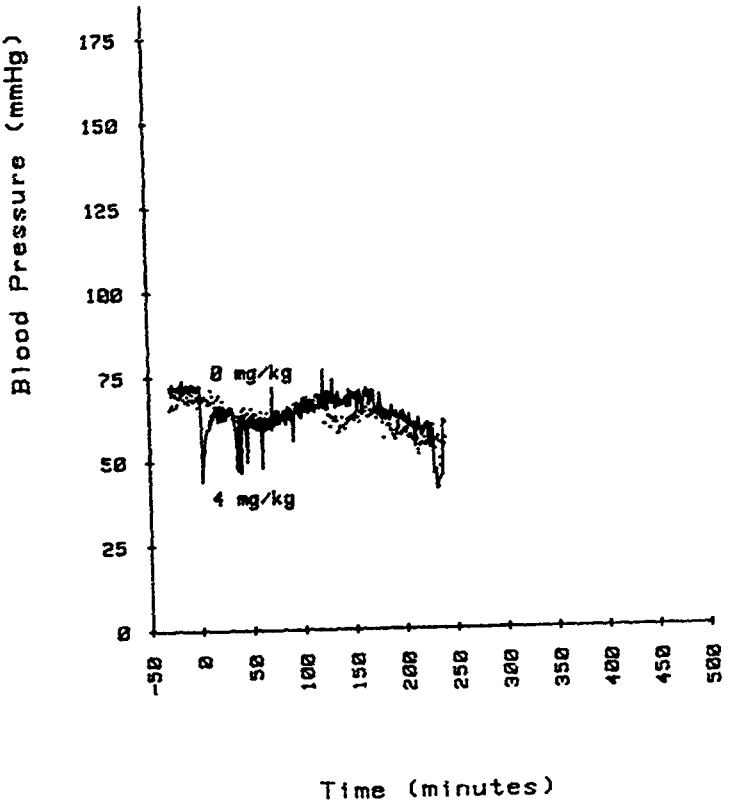


Figure 18. Mean Diastolic Pressure @ 0 & 10 mg/kg

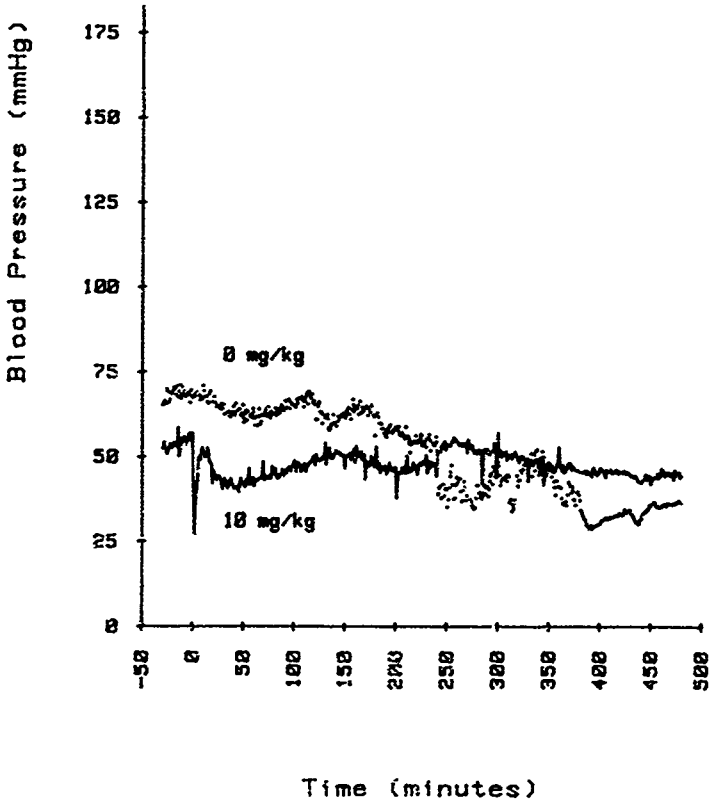


Figure 19. Mean Diastolic Pressure @ 0 & 40 mg/kg

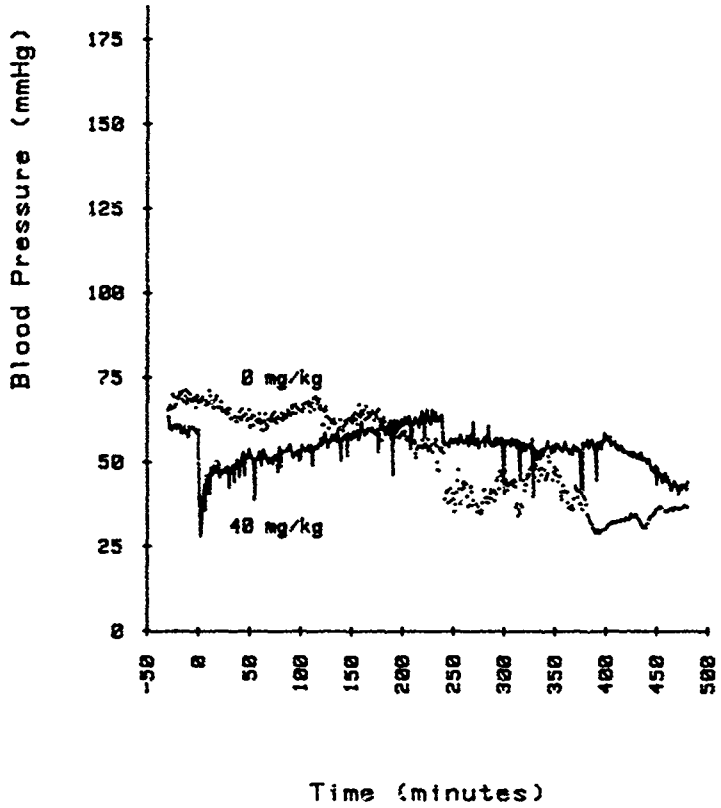


Figure 20. Mean Diastolic Pressure @ 0 & 100 mg/kg

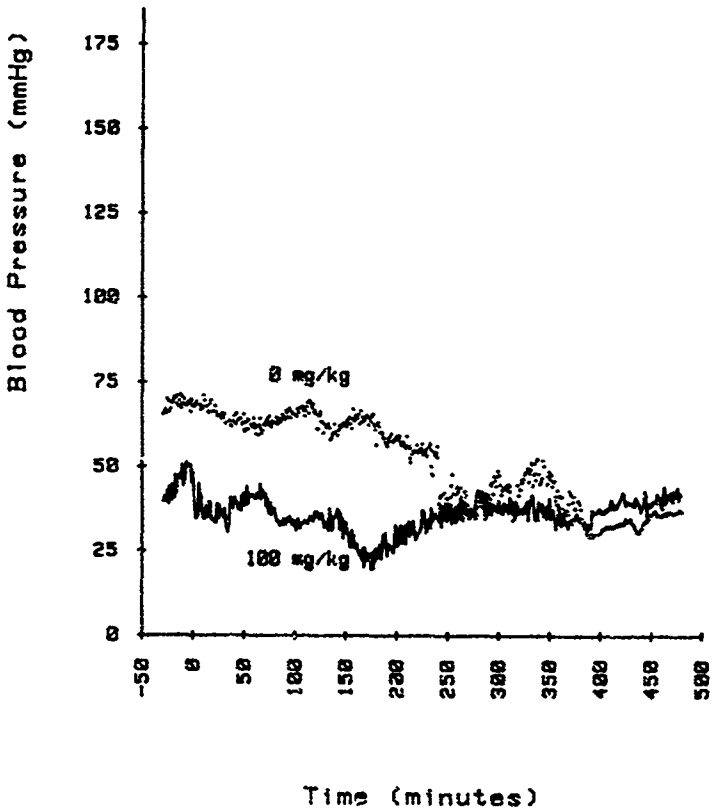
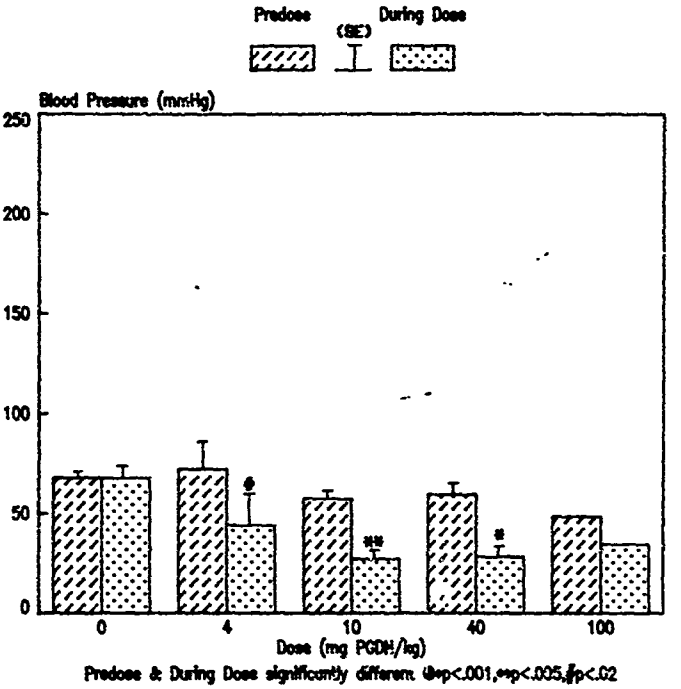


Figure 21. Predose Diastolic Blood Pressure & Minimum Diastolic Blood Pressure During Dosing



4. Pulse Pressure. The pulse pressures were calculated from the diastolic and systolic blood pressure and are plotted (Figures 22 - 25) for each dose level. There is noise introduced into the data due to the aforementioned corrections. The sudden decrease in pulse pressure during PGDN administeration is worth noting. Figure 26 depicts this information more clearly and includes such items as the standard error and the two dose levels (10 and 20 mg/kg) where the decrease is significant.

Since the pulse pressure represents a physiological parameter with a maximum decrease, just as heart rate increases, a Lineweaver-Burk plot was made of the dose and pressure difference data. Figure 27 shows the transformation and from it the Vmax (64.3 mmHg decrease) and Km (5.6 mg/kg) were calculated. The Km value is interesting in that it is very close to the Km for heart rate increase (3.2 mg/kg). It appears that 4 mg/kg was an ideal dose to demonstrate half saturation of these physiological parameters.

5. Urine Output. One of the more surprising findings of the study which should have been considered involved urine production. One usually assumes the autoregulatory mechanisms that maintain adequate pressures in the kidney continue to work even when arterial blood pressures decrease. This apparently is only partially true during PGDN intoxication. Figure 28 illustrates dramatic decreases in urine production as the dose increases. It is theorized that blood pressure and output are cause and effect. At the high dose, urine production had all but ceased even though fluids were given continuously. While the standard error is generally quite high for these mean values, several doses are significantly different from that of the output of the control dogs. The t test used for significance was for independant samples and followed these equations (Ferguson, 1966):

$$S^2 = \frac{\sum_{1}^{N_1} X^2 - (\Sigma X)^2/N_1 + \sum_{2}^{N_2} X^2 - (\Sigma X)^2/N_2}{N_1 + N_2 - 2} \qquad (4)$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S^2/N_1 + S^2/N_2} \qquad (5)$$

Figure 22. Mean Pulse Pressure @ 0 & 4 mg/kg

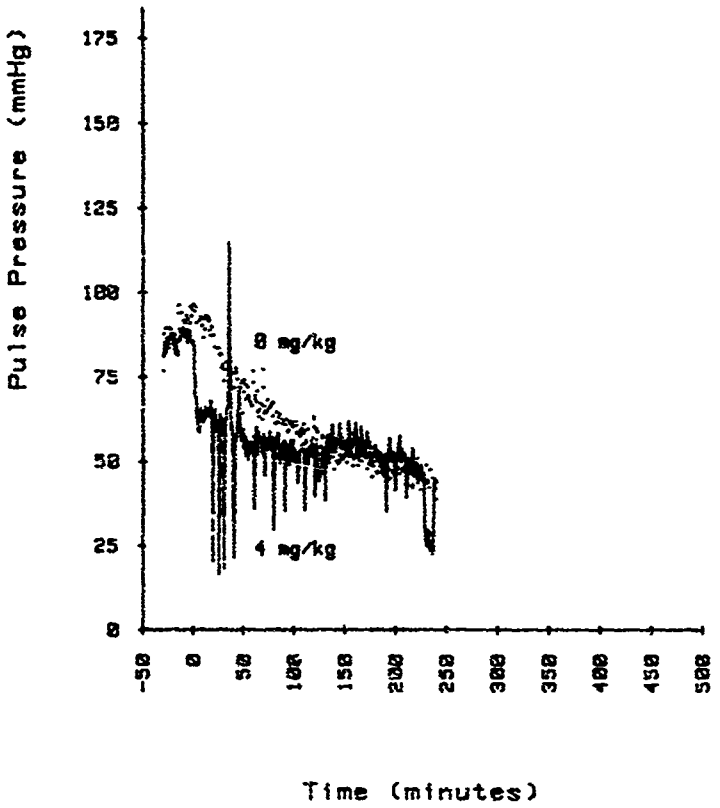


Figure 23. Mean Pulse Pressure @ 0 & 10 mg/kg

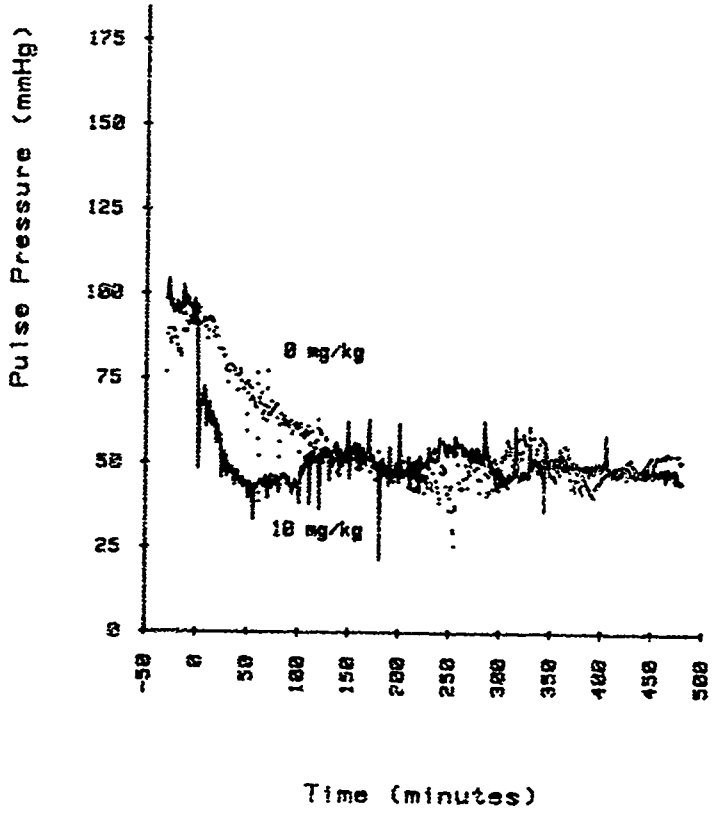


Figure 24. Mean Pulse Pressure @ 0 & 40 mg/kg

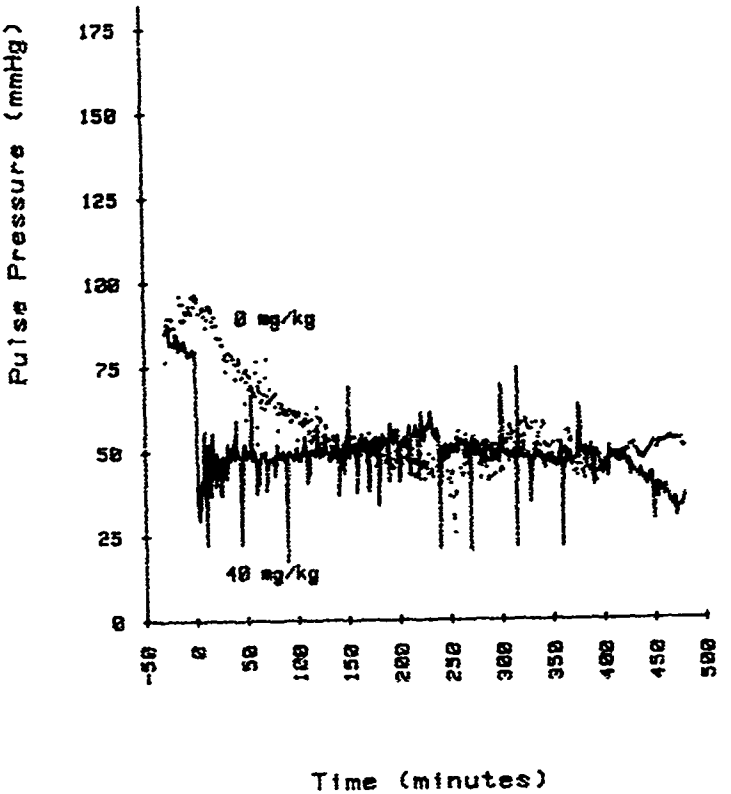


Figure 25. Mean Pulse Pressure @ 0 & 100 mg/kg

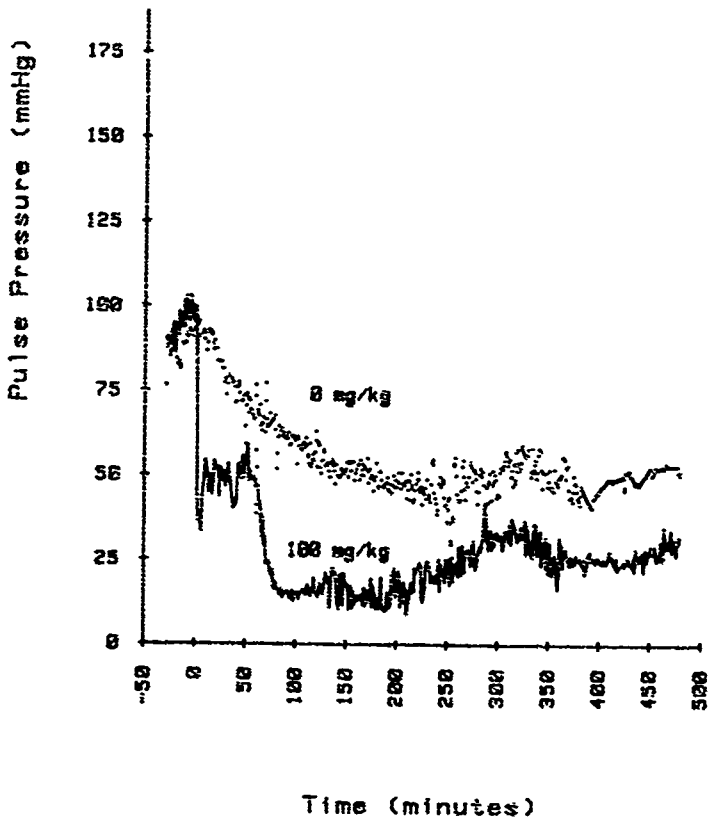


Figure 26. Predose Pulse Pressure & Pulse Pressure During Dosing

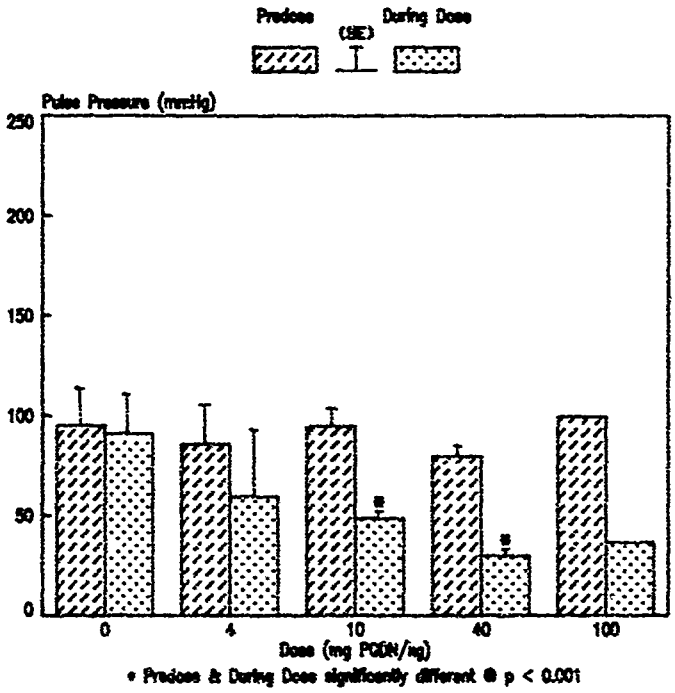


Figure 27. Maximum Pulse Pressure Decrease During Dosing

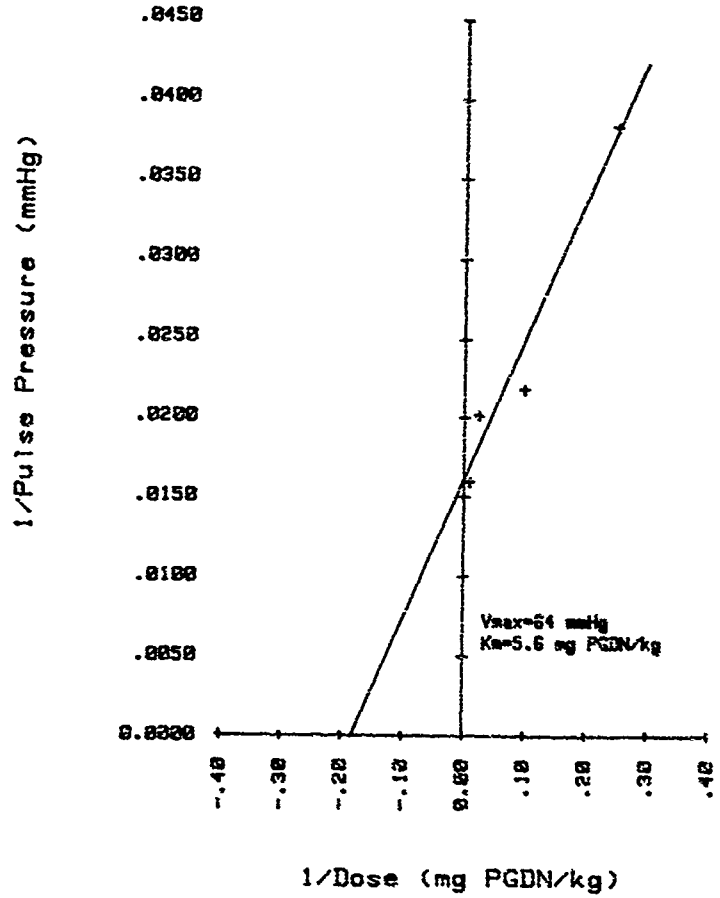
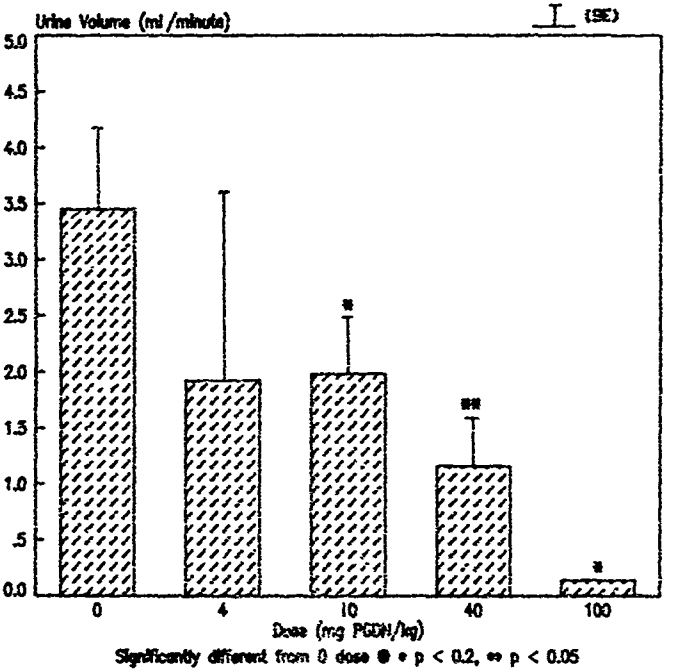


Figure 28. Mean Urine Output After Dosing



Another unexpected finding was the presence of dark brown blood in the urine. This usually coincided with the decrease in output at the start of the experiment and was more severe as the dose of PGDN increased. Blood appearance frequently would stop if urine production resumed as was the case with the animals receiving lower doses. The quantity of blood in the urine precluded it as being an artifact of catheterization.

D. Pathology. Preliminary pathology findings were inconclusive. Gross examination of the dogs revealed some hemorrhage at the mesenteric lymph nodes but only at the frequency listed in Table X.

TABLE X. FREQUENCY OF OCCURRENCE OF HEMORRHAGIC MESENTERIC LYMPH NODES

Dose	0	4	10	40	100
Frequency	0/3	1/2	2/5	1/5	1/1

Light microscopic histopathology of the kidneys, bladder, prostate and ureter revealed no damage that would permit passage of quantities of blood into the urine. This finding must be considered with the fact that all dogs followed for eight hours were refrigerated overnight before post mortem examination. The delay may mask interpretation of any damage.

We hypothesized that PGDN was causing hemolysis and a change in glomerular filtration so that hemoglobin or myoglobin was being expressed into the urine. Tests on Dog A76 (40 mg/kg) indicated no hemolysis. Results of the urinalysis in Table XI also do not support this idea.

E. Methemoglobin. Several in vitro tests were performed on dog blood maintained at 37.5°C with the proper PCO_2 and PO_2 levels. In these tests, PGDN was added at either 10,000 ng/ml blood, 20,000 ng/ml or 50,000 ng/ml and samples taken for MetHb analysis over a period of six hours. The results are shown in Figure 29, along with the expected range of values for nontreated blood. Significant conversion of hemoglobin (Hb) does not occur unless PGDN is at or above 20,000 ng/ml. Andersen and Smith (1973) obtained evidence that this is a direct reaction of Hb and PGDN without need of an enzyme.

During all of the in vivo tests of PGDN, MetHb analysis was performed on blood samples every thirty minutes. The results of

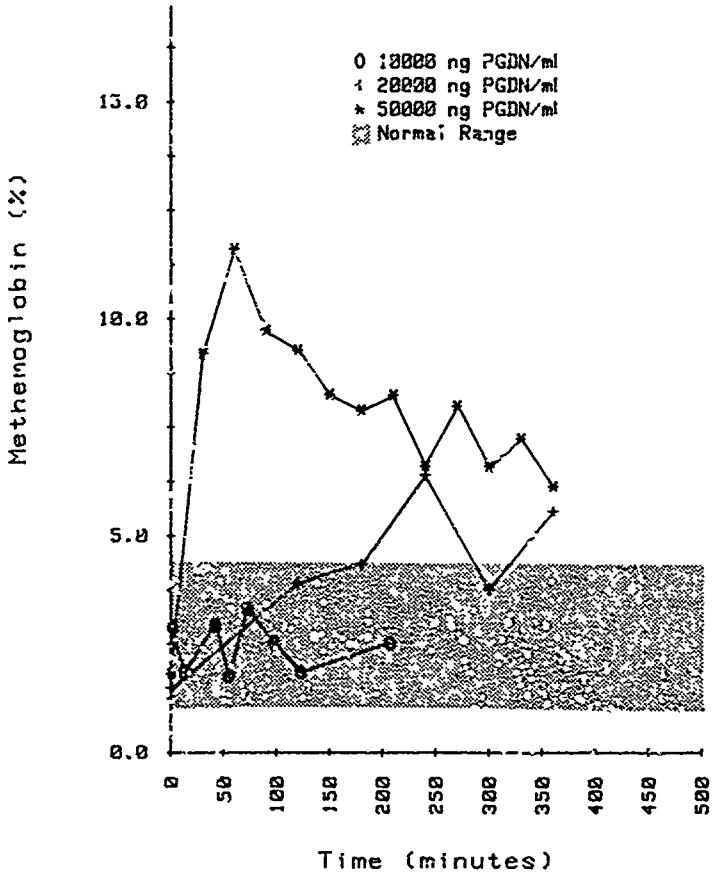
TABLE XI.

URINALYSIS OF DOG A76 (40 mg/kg)

	Time Sample Taken (min).					
	-30	+30	+60	+90	+120	+330
Color	yellow/ cloudy	yellow/ cloudy	tan/ opaque	tan-green/ opaque	yellow/ clear	yellow/ clear
pH	8	8	6	6	6.5	6.5
Protein	Negative	Trace	+1	Trace	Negative	Negative
Occult Blood	Negative	+1	+4	+4	+4	+4
WBC	Occasional	5-9	--	--	--	Occasional
RBC	1-3	150-200	TNTC*	TNTC*	--	Rare
Epithelial Cells	--	3-6	10-20	5-10	Rare	Rare
Glucose	+2	+2	+4	Trace	+4	+2

*TNTC = Too numerous to count

Figure 29. In Vitro Methemoglobin Data



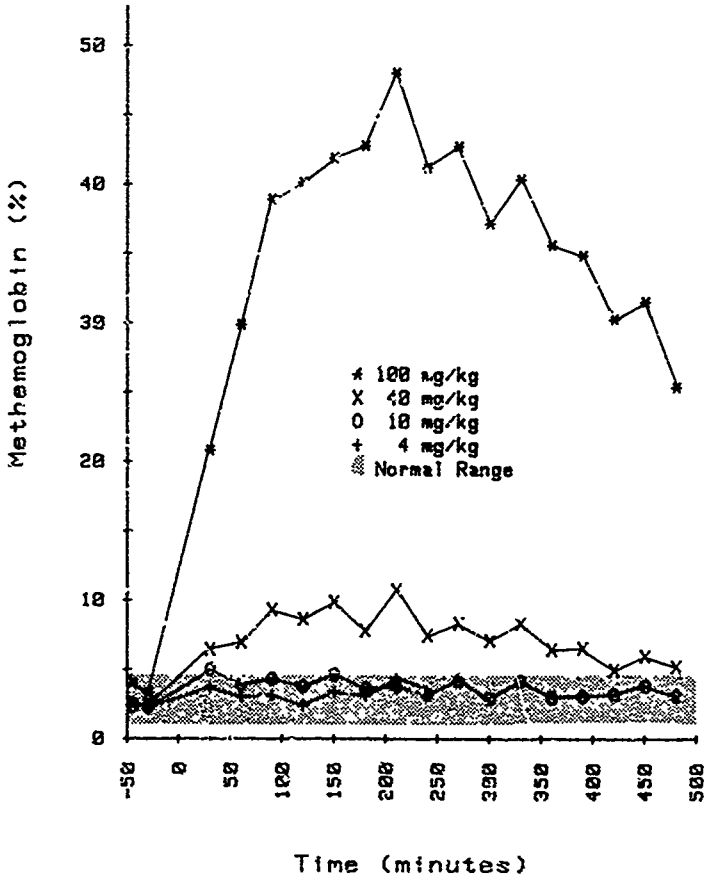
the individual tests are listed in the tables in the appendices. Mean values were calculated for each PGDN group and plotted in Figure 30. Except for one sample collected at 210 minutes, the 4 mg/kg treated dogs were indistinguishable from control values. The mean values for 10 mg/kg dosed dogs in 10 out of 16 post dose sample times are significantly different from control values at $p<0.02$. The 40 mg/kg dose values are all significantly different ($p<0.001$) and similar in magnitude to the methemoglobin values in the 50,000 ng/ml in vitro test. The 100 PGDN mg/kg treated dog had clearly excessive levels of MetHb. As has been noted before, values above 40% can increase heart rate which was the case for this animal. It is surprising that while 40% MetHb has toxic implications, conversions of 60% to 70% have been found to be most effective in treating cyanide poisoning (Kiese, 1974).

P. PGDN Kinetics. 1. Blood In Vivo. Preliminary experiments on intravenous dosing of PGDN in dogs showed that elimination was quite rapid. A sampling schedule was established so that the generated curves of blood concentration versus time would be adequately described. The sampling times were every 5 minutes during the first hour and every 10 minutes during the next three hours.

One animal each was dosed at 0, 4, 10 and 40 mg/kg and followed for four hours. The elimination plots were made and the data treated by stripping, as it appeared that PGDN elimination was a two compartment model (O'Flaherty, 1981). Calculation of the half life in the second compartment showed that the experiments had to be extended to eight hours if the final phase was to be followed for periods covering four to five half lives. This required that samples be taken every fifteen minutes for an additional four hours. The corrected PGDN blood concentrations are listed in the appendix. Sampling of the 4 mg/kg dosed dogs was not extended, since at the end of four hours, the PGDN values were either close to or at the lower limit of detection. The remaining animals were dosed as shown on Table III.

Hand calculation of the data produced terminal half lives of 85 minutes for the 4 mg/kg, 101 minutes for the 10 mg/kg, 128 minutes for the 40 mg/kg and 97 minutes for the 100 mg/kg dose. A computer program was used to determine the equation for the line. This was a nonlinear regression program which iteratively improved upon the initial set of parameters supplied by the curve stripping technique. The program was based on Marquardt's (1963) application of the Gauss-Newton method (Fletcher, 1971). This particular program is designed to reduce the sum of the squares of the

Figure 30. Methemoglobin in PGDN Dosed Dogs



differences between points on a model line and the empirical data by 30% of that achieved by curve stripping (Bulkley et al., 1980).

The two compartment model was applied to blood data for each dog. It was immediately apparent that more than two compartments existed. A three compartment model was devised and each animal's data analyzed by computer. The plot comparing both models using data from one dog (Figure 31) shows that the three compartment model is superior. The three compartment equation is:

$$C = \alpha e^{-k_1 t} + \beta e^{-k_2 t} + \gamma e^{-k_3 t} \qquad (6)$$

where C = blood concentration at time t; α = function relating microscopic rate constant k_1 to multicompartment model; β = function relating microscopic rate constant k_2 to multicompartment model; λ = function relating microscopic rate constant k_3 to multicompartment model.

Mean results of each group's parameters and the mean of all animal data are listed in Table XII. Note that the biological half lives of the terminal phase are quite close to those calculated by stripping. The half-lives and rate constants values point up the proposition that the biophase resides in the central (blood) compartment particularly since there is a relatively instantaneous onset of pharmocodynamic action which decreases rapidly much like the first phase. This is a commonly accepted principle as defined by Garrett (1980) and others.

In order to visualize the equations and the data from which they originated, Figures 32 to 35 were drawn. A combination of the four curves was drawn (Figure 36) for direct comparison. The only curve not closely parallel is that for the 4 mg/kg data which represent only two animals and is more subject to error.

There are a variety of ways in which to estimate the volume of distribution, all of which tend to either over or under estimate the physiological volume of distribution (Gillette, 1974 and O'Flaherty, 1981). Since clearance will eventually be calculated, a volume of distribution of the central compartment [Vd(c)] is the most useful approximation. Parameters from the three compartment equations were used to calculate the Vd(c) using the following equation.

$$Vd(c) = \frac{Do}{\alpha + \beta + \gamma} \qquad (7)$$

TABLE XII.

EQUATION PARAMETERS FOR PGDN
ELIMINATION IN THE DOG

$$C = \alpha e^{-k_1 t} + \beta e^{-k_2 t} + \gamma e^{-k_3 t} \qquad (6)$$

Dose(mg/kg)	α(ng/mL)	$k_1(min^{-1})$	β(ng/mL)	$k_2(min^{-1})$	λ(ng/mL)	$k_3(min^{-1})$
4	52186	0.74886	2527	0.10283	697	0.01201
t 1/2	--	0.9 min	--	6.7 min	--	57.7 min
10	16026	0.31283	3300	0.04207	1042	0.00650
t 1/2	--	2.2 min	--	16.5 min	--	106.6 min
40	162046	0.36084	18812	0.04869	6915	0.00553
t 1/2	--	1.9 min	--	14.2 min	--	125.3 min
100	119643	0.25293	51942	0.04036	35525	0.00639
t 1/2	--	2.7 min	--	17.2 min	--	100.6 min
Mean	--	0.39377	--	0.05383	--	0.00700
S.E.	--	0.06888	--	0.00777	--	0.00080
t 1/2 (biological)	--	1.8 min	--	12.9 min	--	99.0 min

Figure 31. 2 & 3 Compartment Analysis on A76

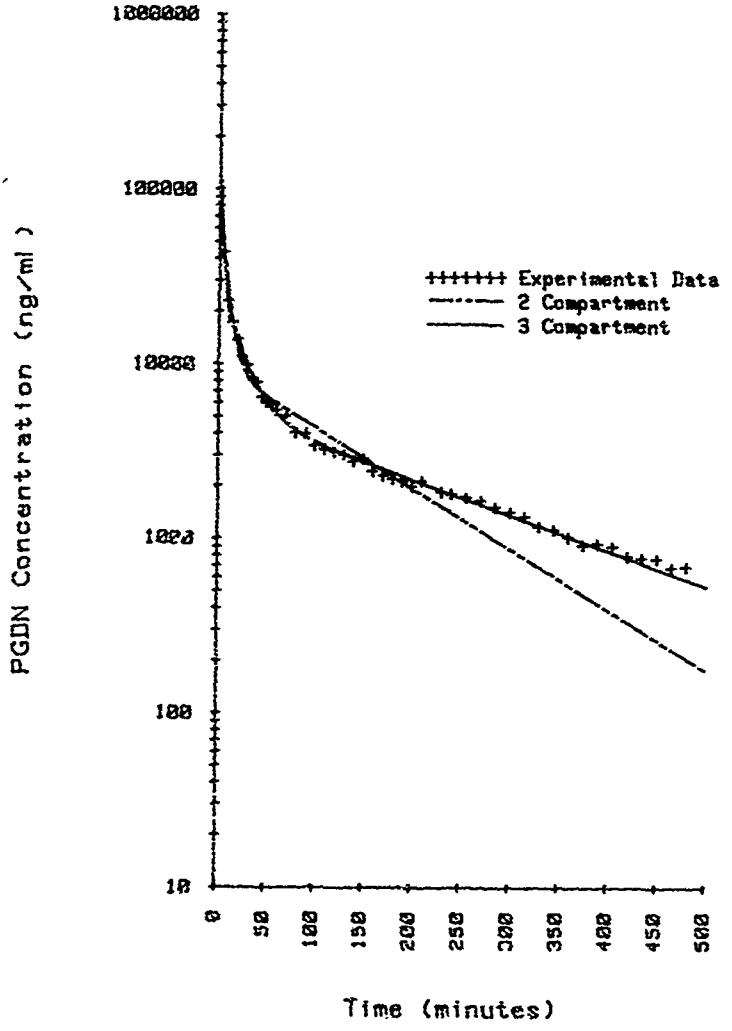


Figure 32. PGDN Blood Data @ 1 mg/kg

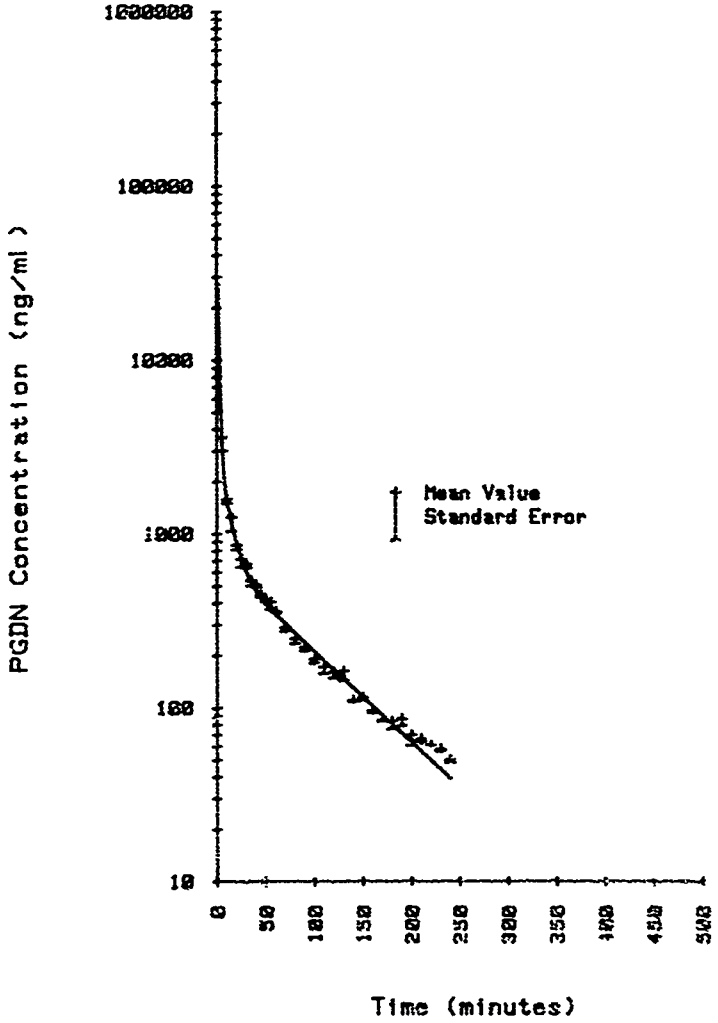


Figure 33. PGDN Blood Data @ 10 mg/kg

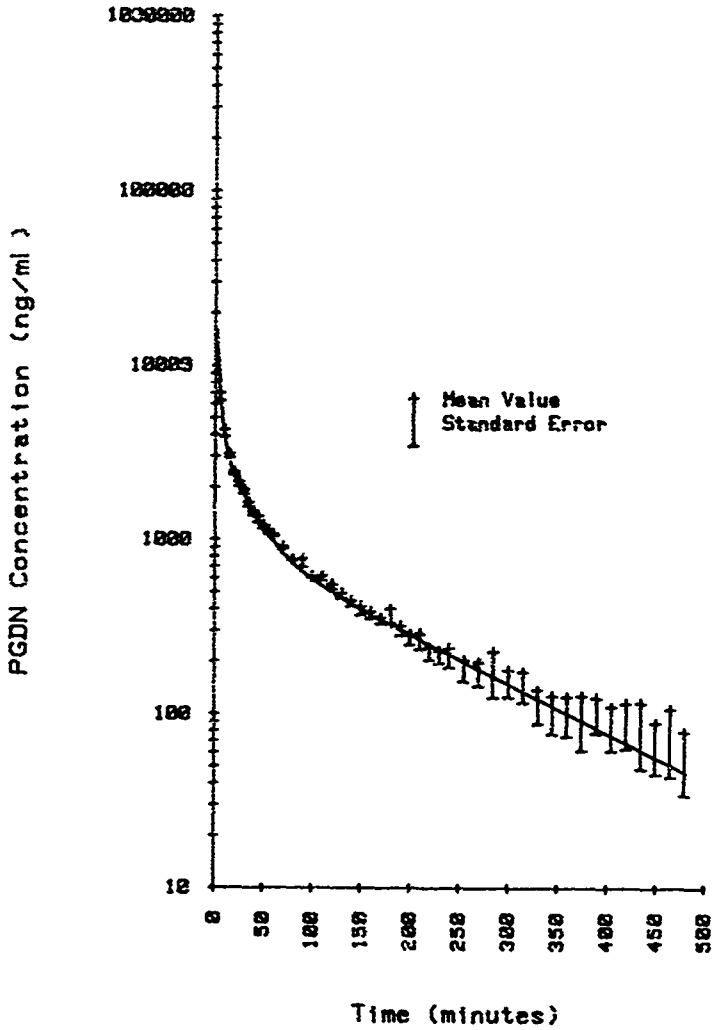


Figure 34. PGDN Blood Data @ 40 mg/kg

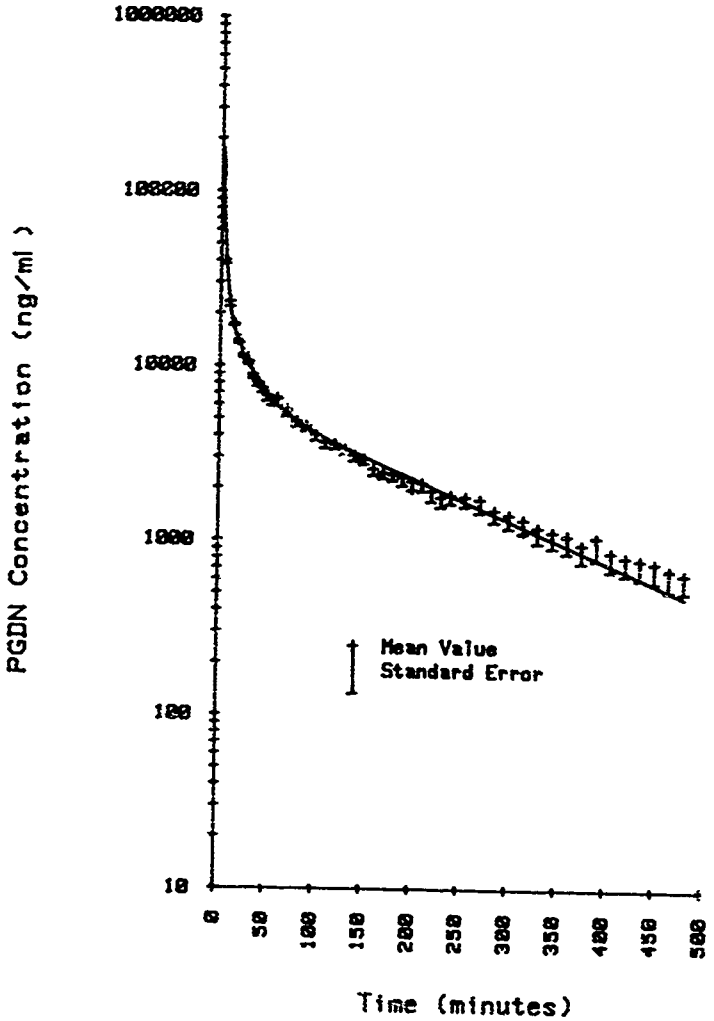


Figure 35. PGDN Blood Data @ 100 mg/kg

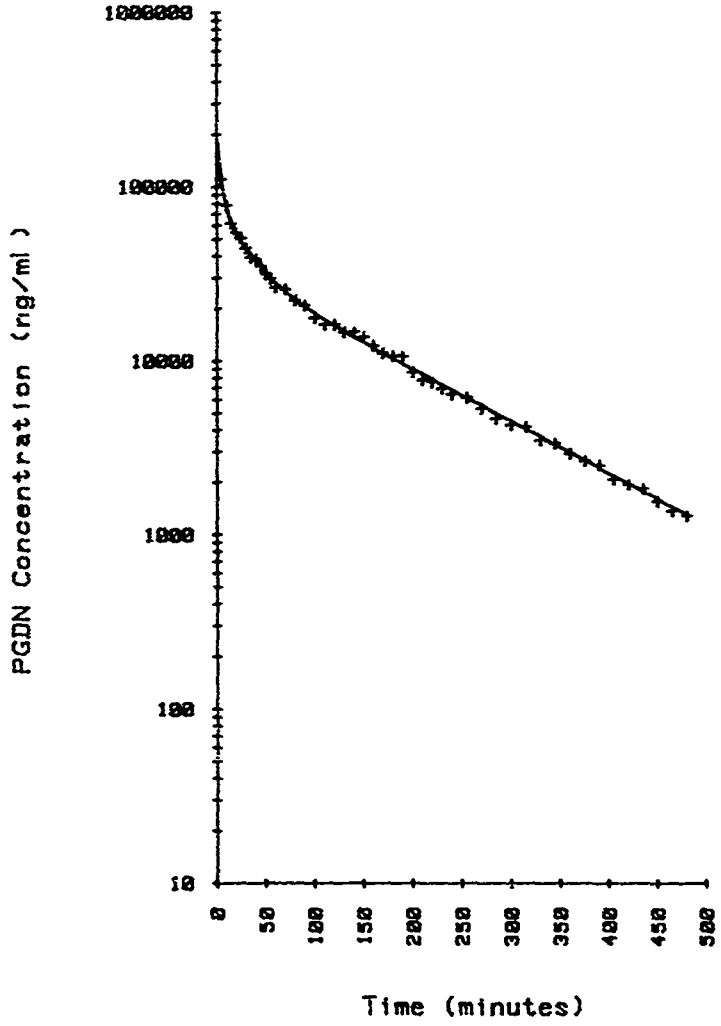
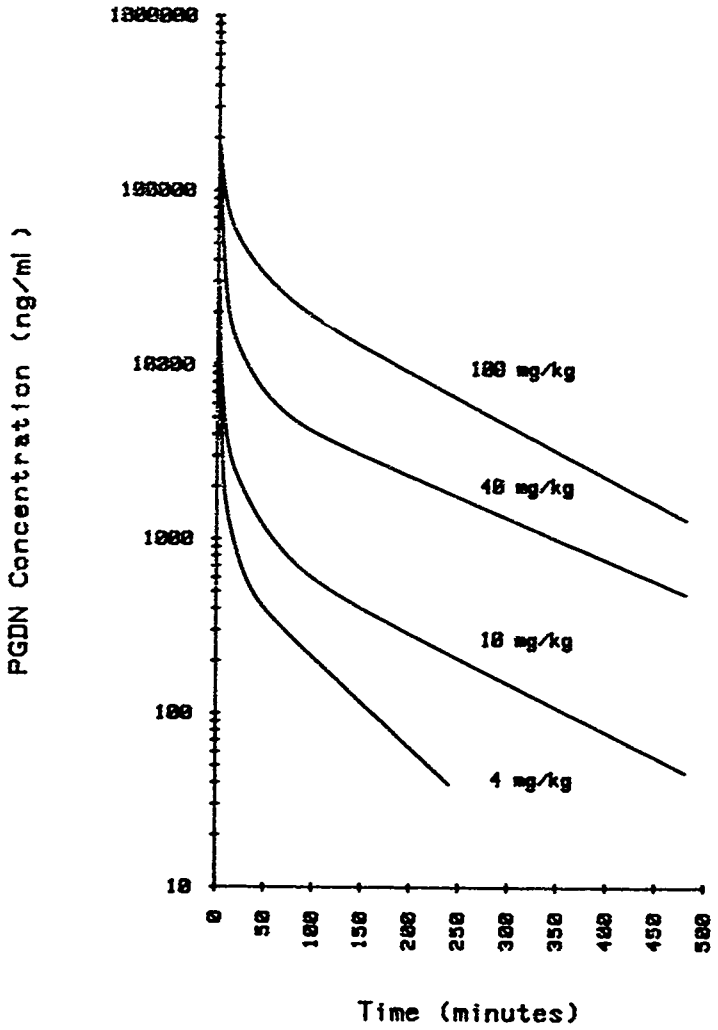


Figure 36. PGDN Blood Elimination Curves



when t = 0 and Do = intravenous dose. The mean values are listed in Table XIII.

TABLE XIII. MEAN VOLUME OF DISTRIBUTION OF THE CENTRAL COMPARTMENT

Dose	Vd(c) (± S.E.) in ml
4 mg/kg	900 (±50)
10 mg/kg	6590 (±1100)
40 mg/kg	4170 (±1020)
100 mg/kg	5860
All Animals	4730 (±780)

2. Blood In Vitro. Several tests were designed and run so that the metabolism of PGDN in the red blood cell could be followed. These in vitro experiments used freshly drawn heparinized whole dog blood. Temperature was maintained at 37.5°C.

The first test was to spike blood at a nominal concentration of 10,000 ng PGDN/ml, shake briefly, withdraw 1 ml samples periodically and analyze for PGDN. Over several hours blood could lose CO_2 and O_2 and pH would change. Therefore, a second test was run at the same starting concentration with gas containing 5.6% CO_2, 12% O_2 and 82.4% N_2 being slowly bubbled through the blood. This gas produced a PCO_2 of 40 mmHg, PO_2 of 100 mmHg and helped maintain pH at 7.4. The results of these two experiments (Figure 37) were essentially the same.

A third test was performed like the second except that the samples were separated into cells and plasma in a refrigerated centrifuge since we had previously determined that red blood cell (RBC) metabolism of PGDN stopped at 4°C. This test was to determine if PGDN would be preferentially associated with cells or plasma. The mean partition coefficient for [RBC]/[Plasma] was 2.12 over the entire sample range. The rate at which the partitioning occurred was too rapid for current analytical techniques to monitor.

Two other experiments were similar to the second but were run at nominal concentrations of 20,000 and 50,000 ng/ml. The data for all five tests are plotted on Figure 38. Two compartment nonlinear regression equations were found appropriate and fitted. These are plotted on Figure 39 and the parameters are listed on Table XIV.

Figure 37. In Vitro PGDN Blood Kinetics

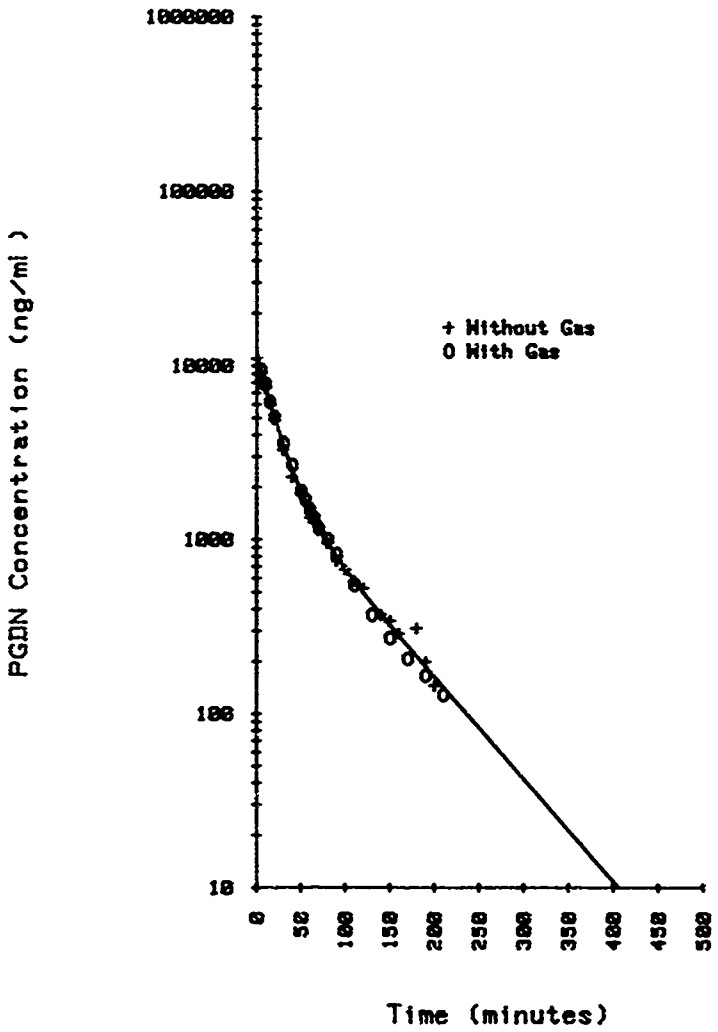


Figure 38. In Vitro PGDN Blood Values

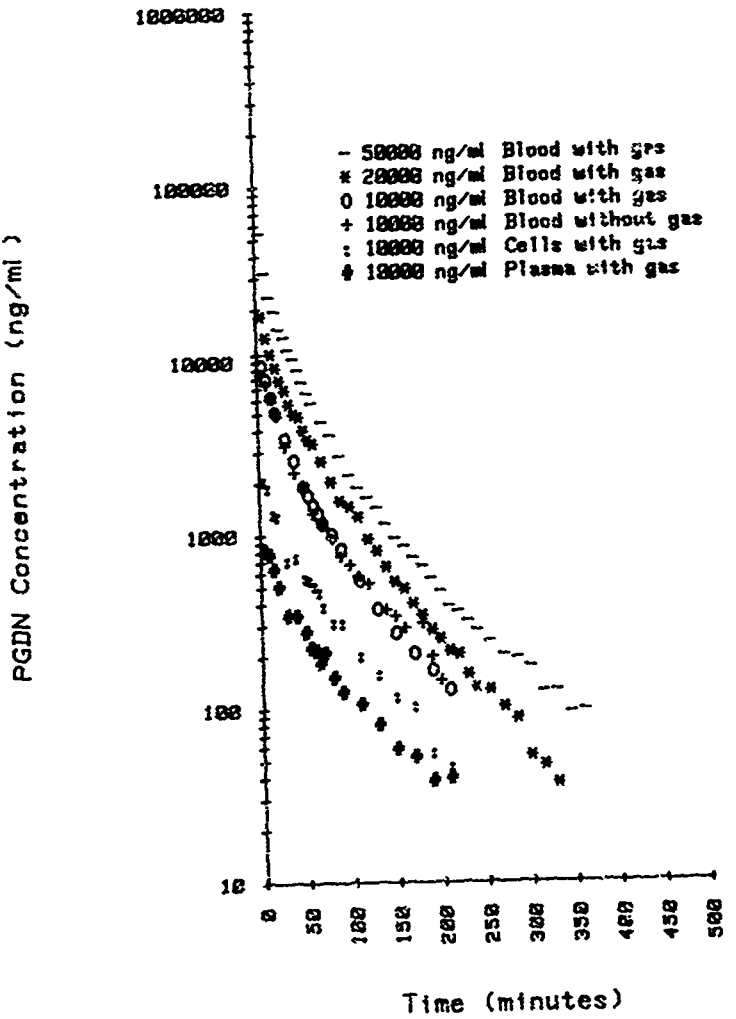


Figure 39. In Vitro PGDN Blood Curves

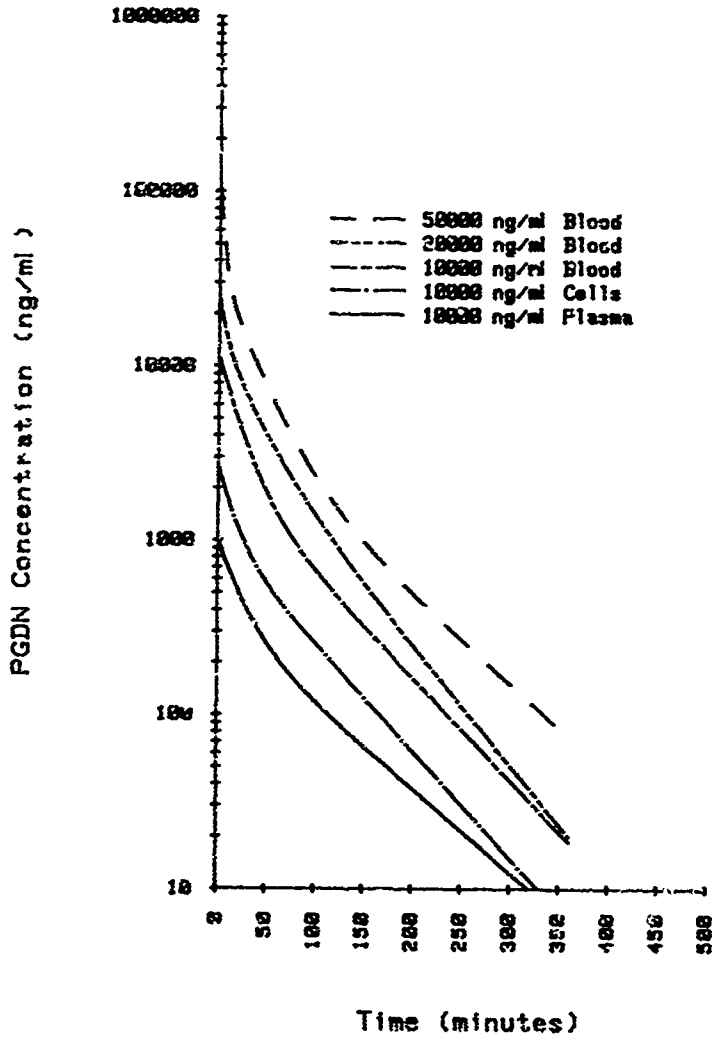


TABLE XIV. TWO COMPARTMENT ANALYSIS OF IN VITRO BLOOD TESTS

$$C = Ae^{-at} + Be^{-bt} \quad (8)$$

Test	A(mg/ml)	a(min^{-1})	B(mg/ml)	b(min^{-1})
Cell	1031	0.06755	1052	0.01416
Plasma	882	0.04757	344	0.01108
10000 ng/ml (no gas)	9303	0.05128	1913	0.01146
10000 ng/ml (gas)	9086	0.05305	2443	0.01556
20000 ng/ml (gas)	13366	0.10236	11078	0.02049
50000 ng/ml (gas)	83222	0.21508	29592	0.02625
Mean	--	0.08953	--	0.01617
t1/2	--	7.7 min.	--	42.9 min.

3. Urine. All urine samples were analyzed for PGDN. The corrected concentrations for each animal are listed in the appendix.

Renal excretion was calculated from the equation:

$$dU/dt = (k_{B,U})(Vd(c))(b) \quad (9)$$

where $k_{B,U}$ is the microscopic rate constant for renal elimination, Vd(c) represents the central compartment volume of distribution and b is the blood concentration (Garrett, 1931). To simplify this equation, dU/dt was plotted against the blood values which were calculated from the three compartment equation at the half time between urine collections. The slope of the linear regression line of these points is then equal to $k_{B,U}$ Vd(c). The plots for each dose group are on Figures 40 to 43. It will be noticed that the data are widely scattered which is due to a wide range in individual urine output. A regression line was calculated for each animal and a mean value determined for each parameter. The mean value for each dose level was then plotted as a line on Figures 40 to 43 and all lines on Figure 44 for comparison. From the corresponding volumes of distribution, $k_{B,U}$ can be determined. These are listed on Table XV.

Figure 40. Urine & Blood Data @ 4 mg/kg

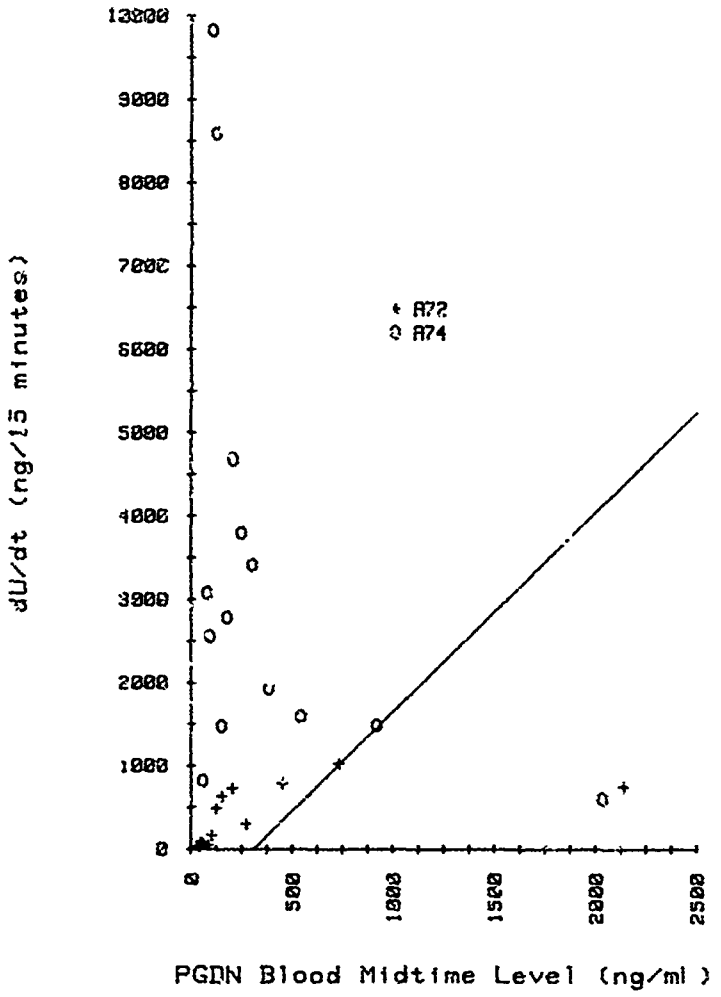


Figure 41. Urine & Blood Data @ 10 mg/kg

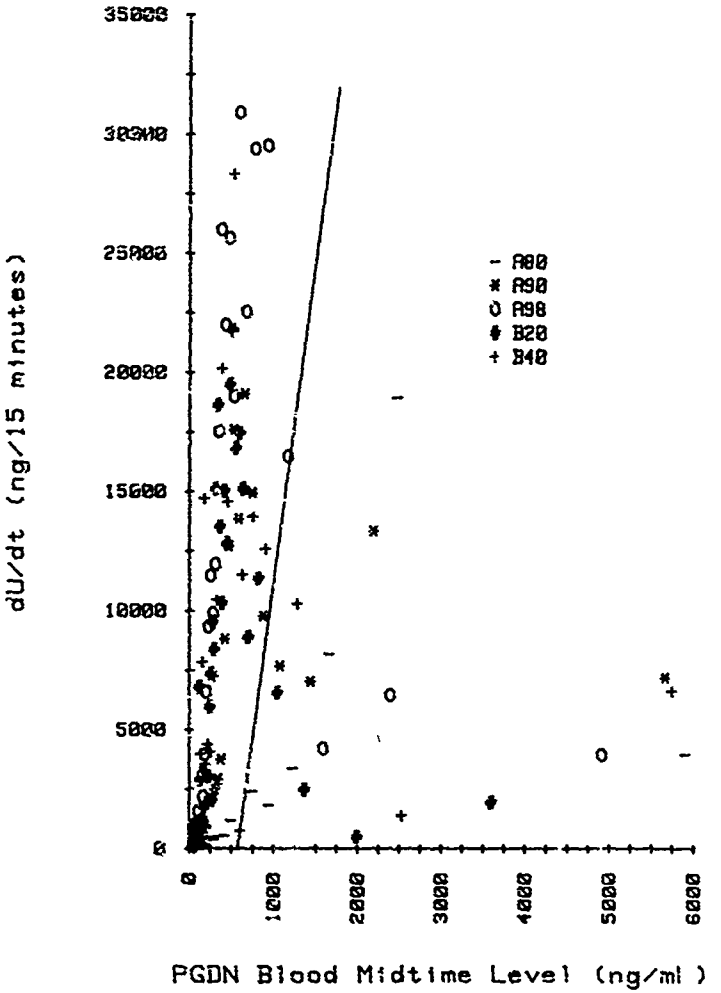


Figure 42. Urine & Blood Data @ 40 mg/kg

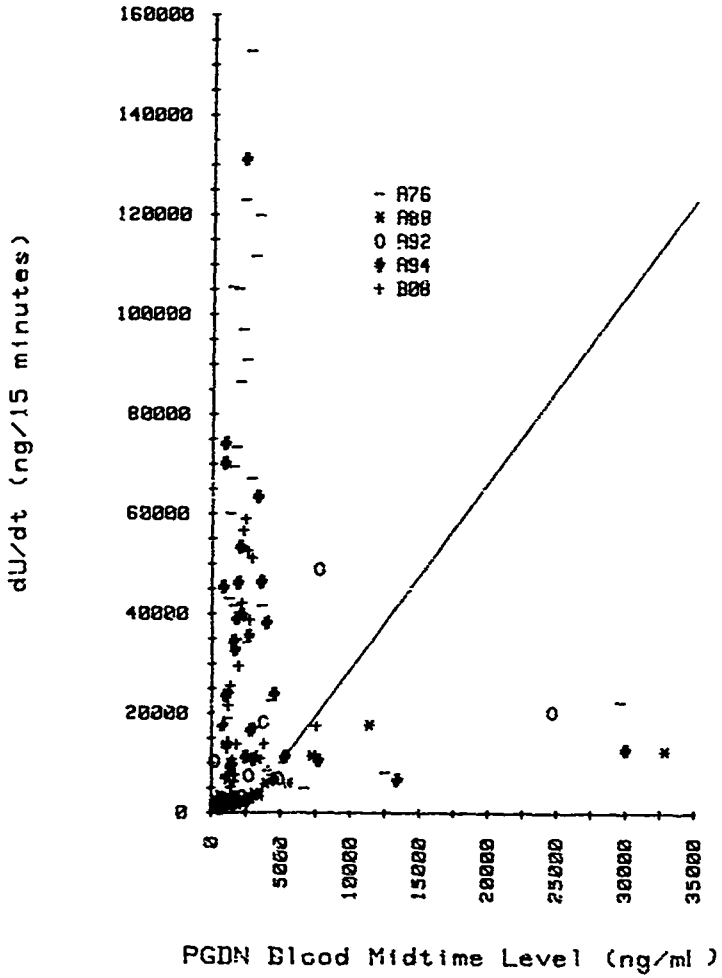


Figure 43. Urine & Blood Data @ 100 mg/kg

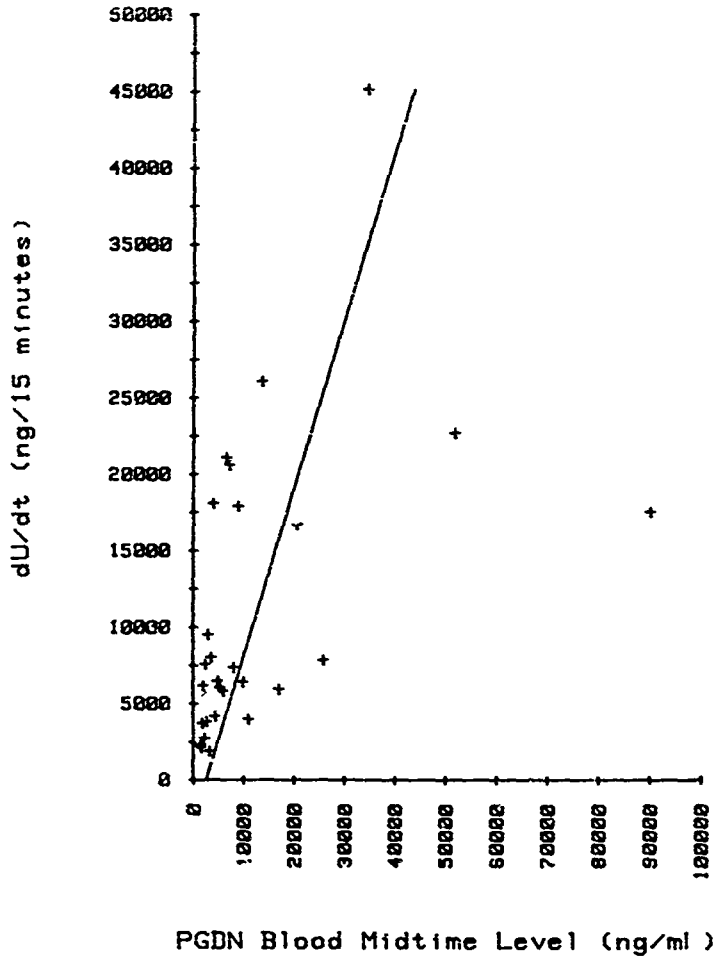


Figure 44. Urine-Blood Elimination Curves

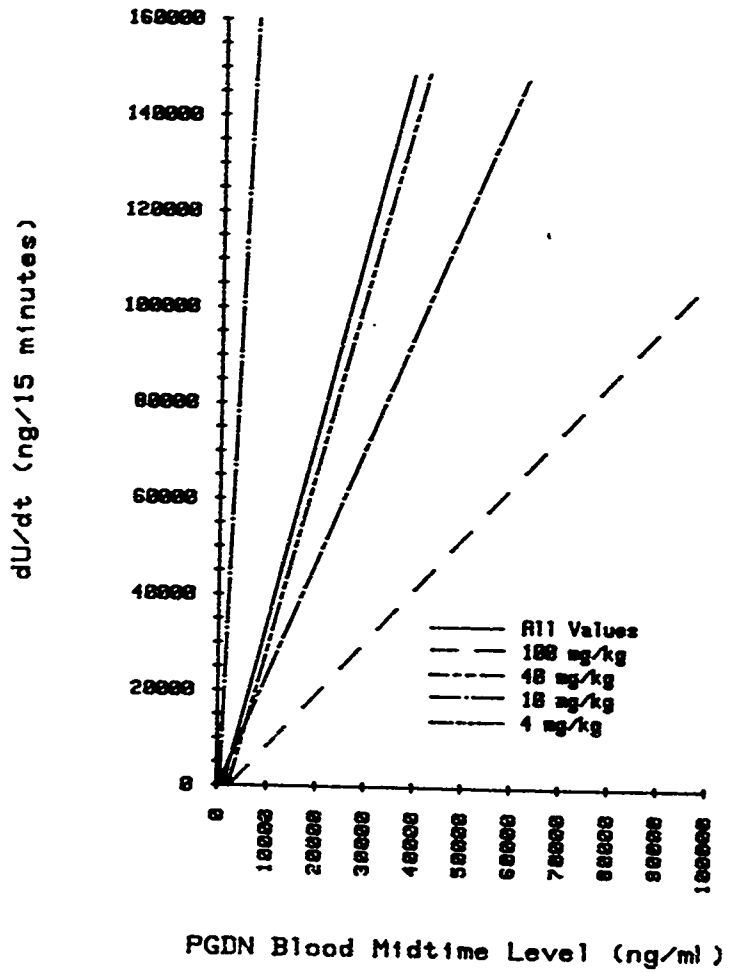


TABLE XV. RENAL ELIMINATION MICROCONSTANTS

Dose	$k_{B,U}(min^{-1})$	% of Dose
4 mg/kg	0.000178	0.049
10 mg/kg	0.000027	0.169
40 mg/kg	0.000060	0.137
100 mg/kg	0.000013	0.025
Mean of all dogs	0.000056	0.127

4. Expired Air. During the last experiment, expired air from the dog was quantified for PGDN by mass spectrometer. Immediately upon the start of dosing, values were recorded. The levels recorded are graphed on Figure 45. After sixty minutes of sampling, no PGDN was detected.

To determine if there was any similarity between expired air and blood values, both were plotted on a time scale (Figure 46). Since diffusion from the pulmonary arterial blood is a first order process, pulmonary clearance should then be like urinary clearance, i.e. the slope of the linear line of rate of excretion in expired air and blood concentration is equal to $k_{B,EA}Vd(c)$. The plot of these relationships is shown on Figure 47. Pulmonary excretion was calculated from the equation:

$$dEA/dt = (k_{B,EA})\ (Vd(c))\ (D) \qquad (10)$$

The microconstant, $k_{B,EA}$ was equal to 0.000149 min^{-1} and the percent of PGDN eliminated in expired air was 0.078%, clearly a minor pathway.

Figure 45. Expired PGDN: Dog Dosed @ 40 mg/kg

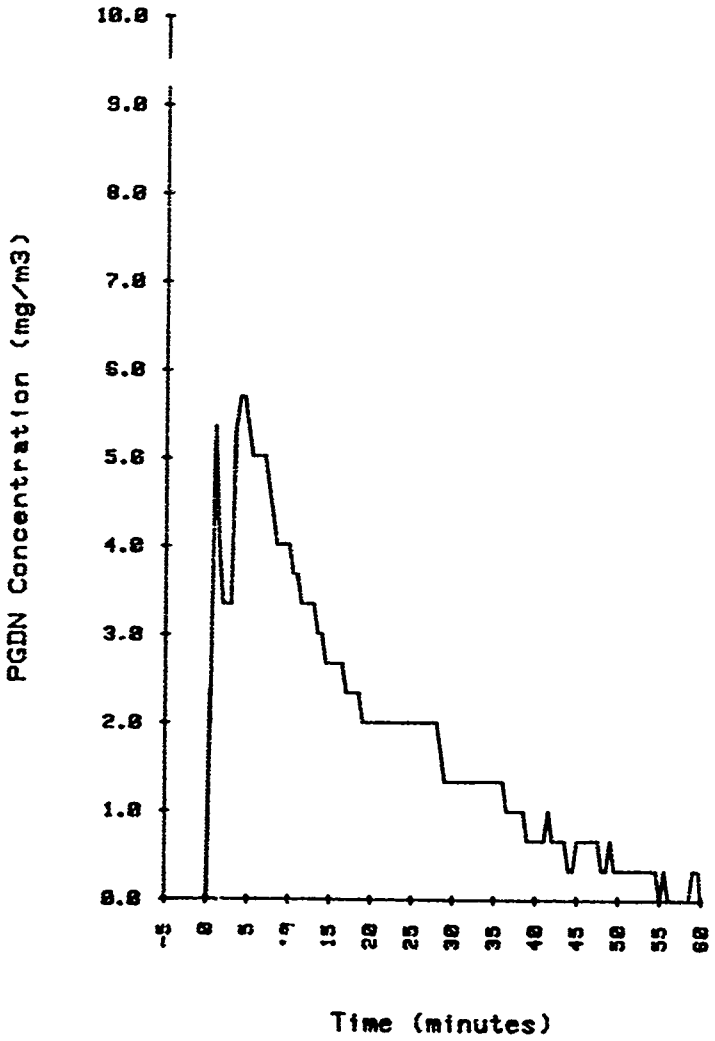


Figure 46. PGDN in Expired Air & Blood A76

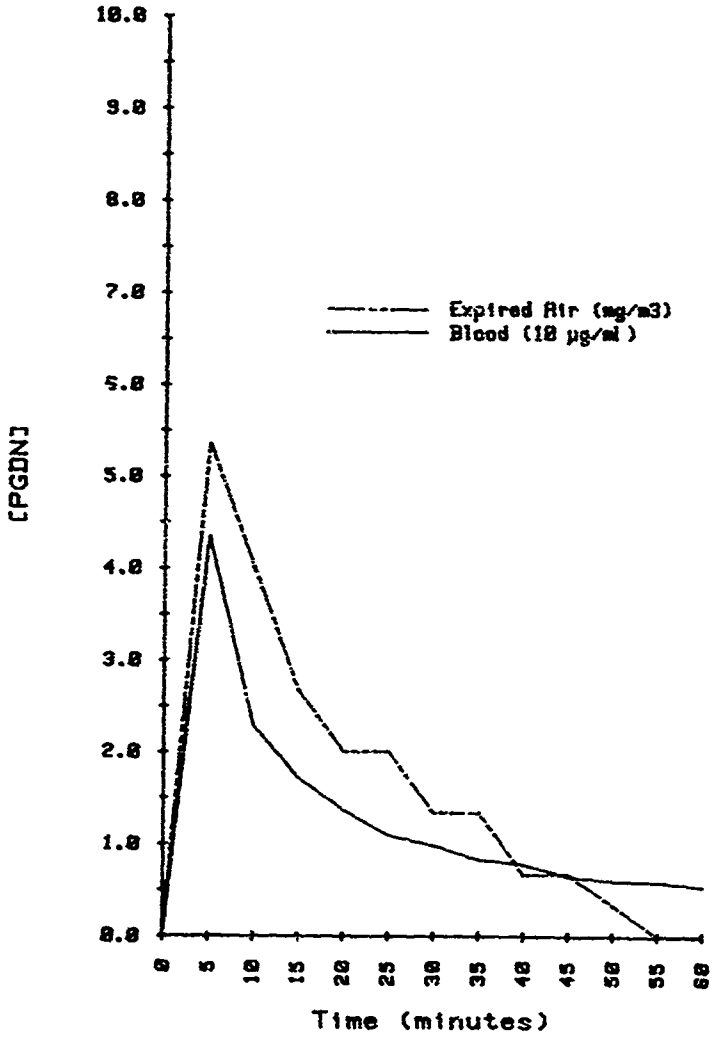
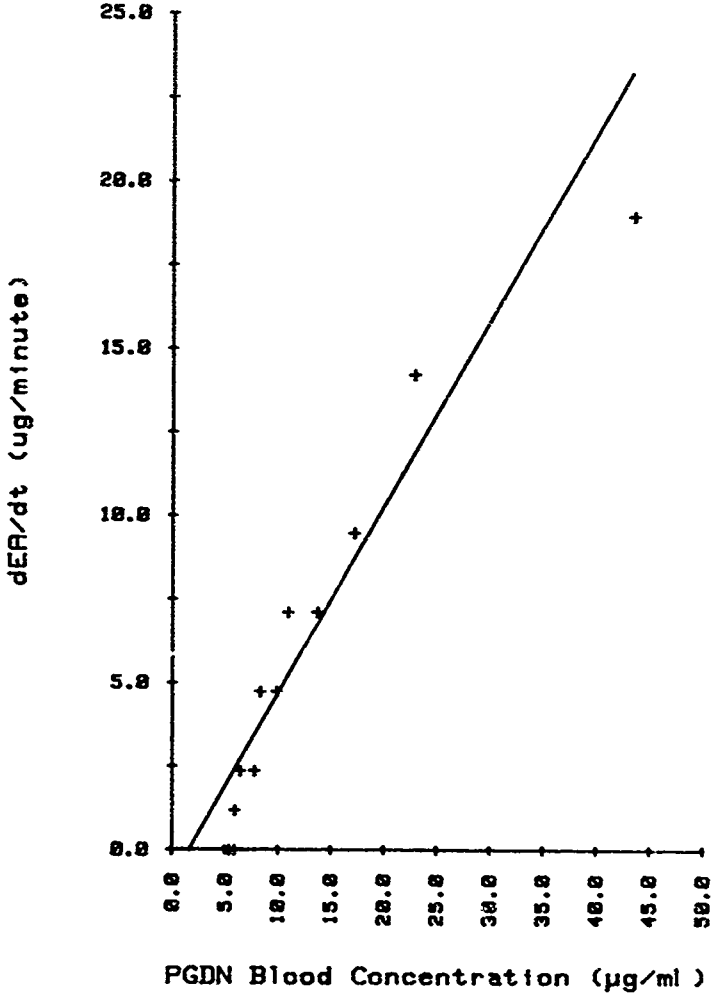


Figure 47. PGDN Expiration Data @ 40 mg/kg



CHAPTER V
COMPARTMENTAL ANALYSIS

A review of the urine, expired air and blood data suggests that a three compartment system best describes the intravenous elimination of PGDN. This is comprised of a central compartment connected reversibly with two "tissue" compartments, one of which is shallow and the other a deep perip eral compartment. The model can be further classified as linear and open since all transfers and eliminations are first order processes from the central area. The losses from the system include urine, expired air and metabolism. A schematic of this model is shown in Figure 48 with the inclusion of all microscopic rate constants necessary to describe the mechanism.

The derivations of the equations describing this model have been solved and published elsewhere and will not be repeated (Gibaldi and Perrier, 1975 and O'Flaherty, 1981). The terminology was modified to fit the PGDN system for which there were seven pertinent equations necessary to determine the microconstants.

$$C = \alpha e^{-k_1 t} + \beta e^{-k_2 t} + \gamma e^{-k_3 t} \qquad (6)$$

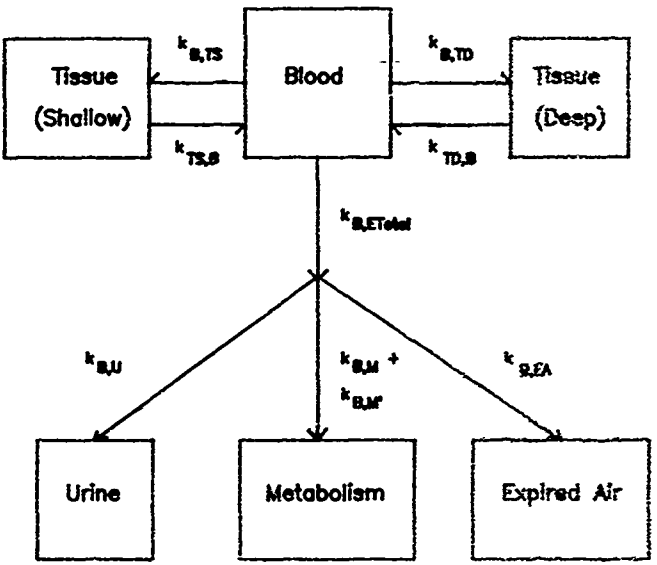
$$\alpha = \frac{Do(k_{TS,B}-k_1)\ (k_{TD,B}-k_1)}{Vd(c)\ (k_1-k_2)\ (k_1-k_3)} \qquad (11)$$

$$\beta = \frac{D_o\ (k_{TS,B}-k_2)\ (k_2-k_{TD,B})}{Vd(c)\ (k_1-k_2)\ (k_2-k_3)} \qquad (12)$$

$$\gamma = \frac{D_o\ (k_{TS,B}-k_3)\ (k_{TD,B}-k_3)}{Vd(c)\ (k_2-k_3)\ (k_1-k_3)} \qquad (13)$$

$$K_{B,E\ Total} = \frac{D_o}{Vd(c)\ (AUC)} \qquad (14)$$

Figure 48. Three Compartment, Linear, Open System



$$k_{B,TS} = \frac{(k_2 \cdot k_3 + k_2 \cdot k_1 + k_3 \cdot k_1) - (k_{TS,B})(k_1 + k_2 + k_3)}{k_{TD,B} - k_{TS,B}}$$

$$- \frac{(k_{B,E\ Total})(k_{TD,B}) + k^2_{TS,B}}{k_{TD,B} - k_{TS,B}} \quad (15)$$

$$k_1 + k_2 + k_3 = k_{B,E\ Total} + k_{B,TS} + k_{B,TD} + k_{TS,B} + k_{TD,B} \quad (16)$$

The mean results of these calculations are in Table XVI.

TABLE XVI. MEAN MICROCONSTANTS FOR THREE COMPARTMENT LINEAR, OPEN SYSTEM

Dose (mg/kg)	$k_{B,TS}$ (min^{-1})	$k_{TS,B}$ (min^{-1})	$k_{B,TD}$ (min^{-1})	$k_{TD,B}$ (min^{-1})	$k_{B,E\ Total}$ (min^{-1})
4	0.1706	0.1342	0.1806	0.0187	0.3596
10	0.1205	0.0960	0.0666	0.0128	0.0656
40	0.1255	0.0917	0.0957	0.0129	0.0893
100	0.1239	0.0970	0.0304	0.0190	0.0300
Overall (±S.E.)	0.1283 (0.0233)	0.1024 (0.0091)	0.09254 (0.0203)	0.0142 (0.0012)	0.1169 (0.0336)

The microconstant $k_{B,E\ Total}$ was calculated from the area under the blood elimination curves. This value of 0.1169 min^{-1} was within 10% of that determined by the addition of the microconstants for urine, metabolism and expired air (0.1059 min^{-1}) which could not be considered as complete since expired air was analyzed on one animal only.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Bolus injections of PGDN were given intravenously to sixteen beagle dogs at one of five doses, including 0, 4, 10, 40 and 100 mg/kg. The animals had been previously anesthetized with α-chloralose so that direct pressure measurements could be made from a femoral artery as well as obtaining numerous blood samples for PGDN, methemoglobin and blood gas determinations. Heart rate and urinary PGDN values were also recorded. The dogs were temperature maintained and mechanically resuscitated to keep P_aO_2 and P_aCO_2 within reasonable limits.

The greatest dynamic effects attributable to PGDN occurred within the fast distribution phase in the central blood compartment. These effects were decreased systolic, diastolic and pulse pressure. They were dose related and returned to control values within several hours. A maximum decrease in pulse pressure of 64.3 mmHg was determined from a Lineweaver-Burk transformation.

Heart rate reflexively increased in response to the vasodilation and this also was dose related. A Lineweaver-Burk plot of the data produced a maximum increase of 129.5 beats per minute. We also found that heart rate slowly increased as a result of the deepening of anesthesia and occurred in control as well as dosed animals.

MetHb values were plotted against time. These percentages ranged from levels that were not significantly different from controls (the 4 mg/kg dose) to those (>40%) which caused an increase in heart rate (the 100 mg/kg dose).

Urine production was adversely affected by PGDN. An additional sequel was the discovery of whole red blood cells in the urine which became more severe as output declined. Histopathology was unable to pinpoint any site of damage which could be traced to the blood problem.

Nonlinear regression methods were applied to the PGDN blood analyses developed for the study. These led to proposing a three compartment, linear open model for the description of PGDN elimination. The equation representing the central compartment was

$$C = \alpha e^{-0.39377t} + \beta e^{-0.05383t} + \gamma e^{-0.007t} \qquad (17)$$

Microscopic rate constants were determined for the two reversible peripheral compartments. These were $k_{B,TS} = 0.1283\ min^{-1}$, $k_{TS,B} = 0.1024\ min^{-1}$, $k_{B,TD} = 0.0925\ min^{-1}$ and $k_{TD,B} = 0.0142\ min^{-1}$. The overall elimination rate constant was determined from the area under the curve calculated for ten times the half-life of the terminal phase. This value, $k_{B,E} = 0.1169\ min^{-1}$ was within 10% of the sum of the individual microconstants derived for urine elimination ($k_{B,U} = 0.0000562\ min^{-1}$), lung elimination ($k_{B,EA} = 0.000149\ min^{-1}$) and blood metabolism of PGDN ($k_{B,M} = 0.8953\ min^{-1}$ and $k_{B,M'} = 0.01617\ min^{-1}$).

After taking all of the above into consideration, it appears that major toxic effects are related to the vasodilation properties of PGDN which last only a short time and require significant doses. Delayed drug response includes the effect of methemoglobinemia which is significant only at extremely high PGDN doses. An unresolved problem was the occurrence of blood in the urine. Future studies should address this by investigating renal blood flow and electron microscopic examination of the kidney apparatus. Further efforts should also be directed to improving the rate constants for both expired air and urine elimination even though they contribute less than 1% of the total elimination.

APPENDIX

TABLE XVII.
EXPERIMENTAL DATA FOR DOG A34
Body Weight: 10.21 kg
PGDN Dose - 4 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
						10.0	3.15
-45	36	114	7.35			11.5	2.41
-30	35	107	7.38			15.0	
-15	38	99	7.34			8.0	
0							
5							
10						7.0	
15							
20							
25							
30	44	94	7.26			6.0	
35							
40						11.0	
45							
50							
55							
60	39	102	7.29			35.0	3.48
70						15.0	
75							
80						34.0	
90	39	101	7.30				
100						34.0	
105							
110						57.0	2.45
120	38	103	7.39				
130						53.0	
135							
140							
150	38	101	7.30			46.0	
160						23.0	
165							
170							
180	42	99	7.27			31.0	3.87
190						42.0	
195							

TABLE XVII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
200							
210	39	101	7.29			55.5	
220							
225						53.0	
230							
240						48.0	3.03
255							
270							
285							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in Urine

Figure 49. Rectal Temperature, Heart Rate and Blood Pressure of Dog A34

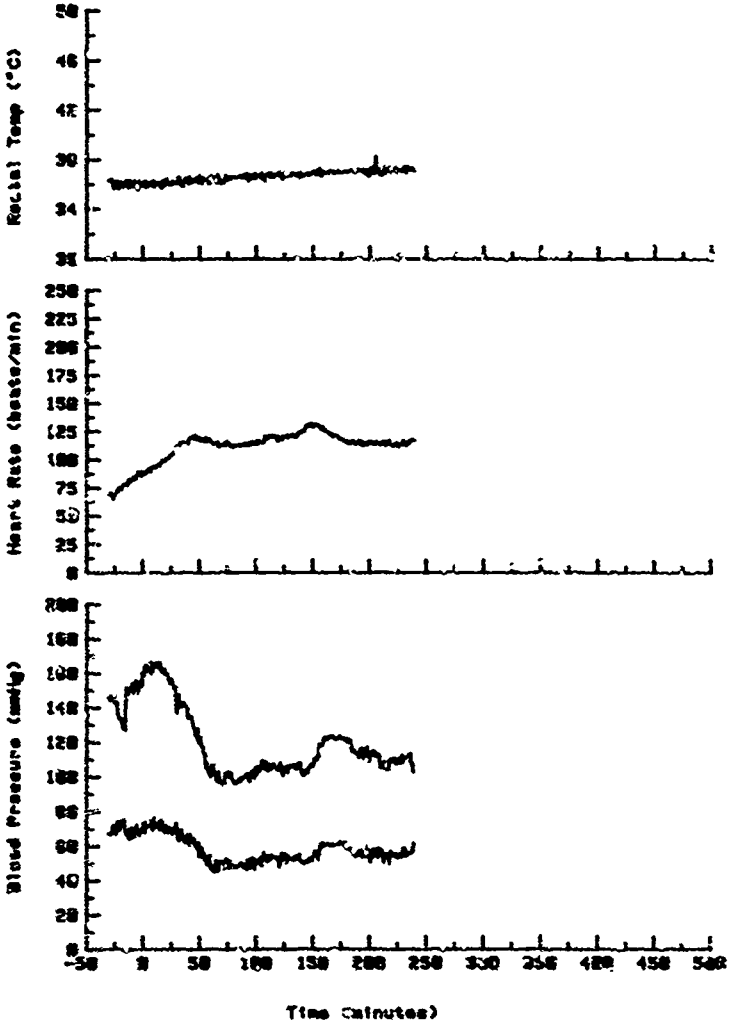


TABLE XVIII.
EXPERIMENTAL DATA FOR DOG A96
Body Weight: 9.75 kg
PGDN Dose - 4 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	38	109	7.25				1.75
-30							1.79
-15	36	105	7.33				
0						18.5	
5							
10							
15						3.8	
20							
25							
30	46	87	7.27			6.8	2.18
35							
40							
45	37	106	7.32			12.2	
50							
55							
60	40	100	7.28			41.5	2.94
70							
75						68.0	
80							
90						115.5	6.21
100							
105	42	87	7.28			107.0	
110							
120	44	95	7.27			117.0	3.07
130							
135						135.0	
140							
150	39	103	7.30			100.0	3.81
160							
165						141.0	
170							
180						64.0	3.76
190							
195	37	107	7.30			92.0	

TABLE XVIII. (Continued)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
200							
210	35	105	7.35			69.5	4.20
220							
225						31.0	
230							
240						46.0	2.41
255							
270							
285							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in Urine

Figure 50. Rectal Temperature, Heart Rate and Blood Pressure of Dog A96

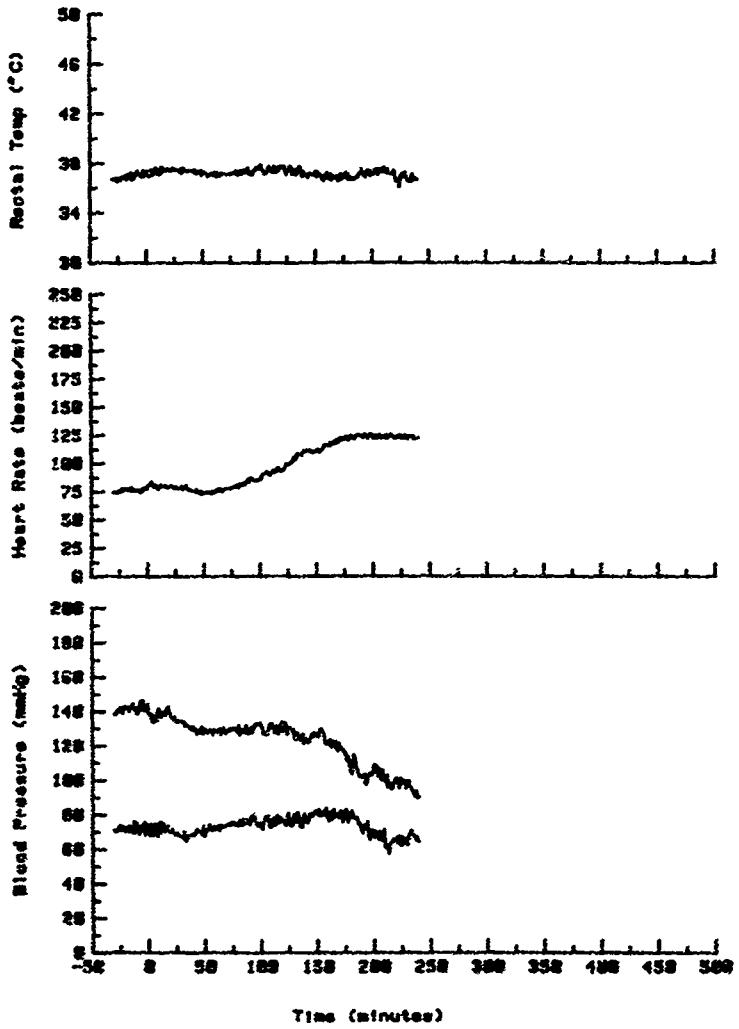


TABLE XIX.
EXPERIMENTAL DATA FOR DOG A68
Body Weight: 12.25 kg
PGDN Dose - 4 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	46	76	7.30			8.0	2.52
-30	39	97	7.35				1.61
-15	40	97	7.35			14.0	
0						4.5	
5							
10							
15						10.5	
20							
25							
30	43	96	7.33			14.0	2.62
35							
40							
45						49.5	
50							
55							
60	42	99	7.35			132.0	2.72
70							
75						103.0	
80							
90	42	97	7.33			158.5	
100							
105						140.0	
110							
120	41	99	7.34			159.0	3.50
130							
135						89.0	
140							
150	39	101	7.34			95.0	
160							
165						79.0	
170							
180	39	100	7.36			87.0	2.31
190							
195						69.0	
200							

TABLE XIX. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	%MetHb (%)
210	40	100	7.36			60.0	
220							
225						51.5	
230							
240	38	100	7.37			16.0	2.37
255						3.0	
270	38	100	7.38			11.5	
285						20.0	
300	41	97	7.38			13.5	3.42
315						18.0	
330	43	96	7.33			36.0	
345						59.0	
360	43	99	7.36			38.6	
375						14.0	
390	42	104	7.36			5.0	
405						3.0	
420	40	109	7.38			13.0	
435						5.0*	
450	44	104	7.37			0.0	
465						0.0	
480	43	104	7.39			22.0	

* Blood in urine

Figure 51. Rectal Temperature, Heart Rate and Blood Pressure of Dog A68

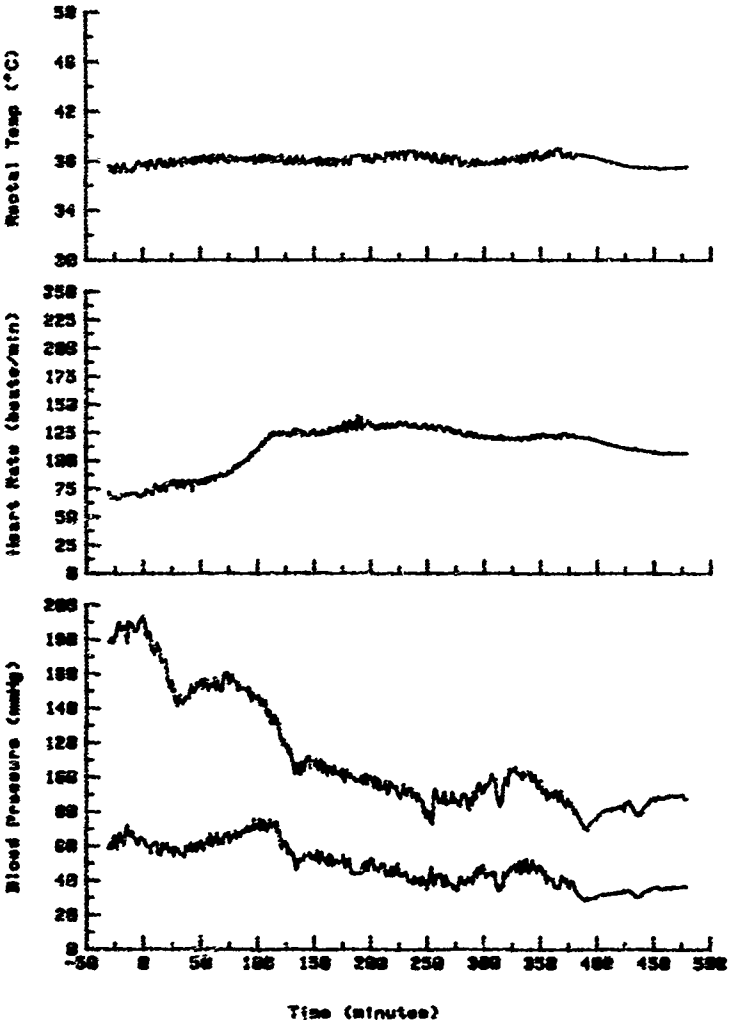


TABLE XX.
EXPERIMENTAL DATA FOR DOG A72
Body Weight. 11.37 kg
PGDN Dose - 4 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45							2.06
-30	36	107	7.32				2.40
-15	38	105	7.35				
0						6.2	
5				4141			
10				1498			
15				1029	298	2.5	
20				810			
25				646			
30	39	96	7.30	638	270	3.8	3.62
35				503			
40				499			
45				435		0.0	
50				412			
55				444			
60	41	99	7.32	369	393	2.0*	2.5
70				305			
75						3.2*	
80				265			
90	37	103	7.35	234	137	2.2	3.19
100				200			
105						0.0	
110				186			
120	37	105	7.35	171	215	3.4	2.38
130				144			
135					96	6.6	
140				109			
150	38	105	7.33	119	123	4.0	2.98
160				94			
165					47	3.5	
170				84			
180	36	104	7.35	75	24	2.1	2.92
190				94			
195						1.4	

TABLE XX. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
200				61			
210	37	101	7.34	64	23	2.8	5.50
220				61			
225					23	3.5	
230				59			
240				48	13	2.4	4.26
255							
270							
285							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in urine

Figure 52. Rectal Temperature, Heart Rate and Blood Pressure of Dog A72

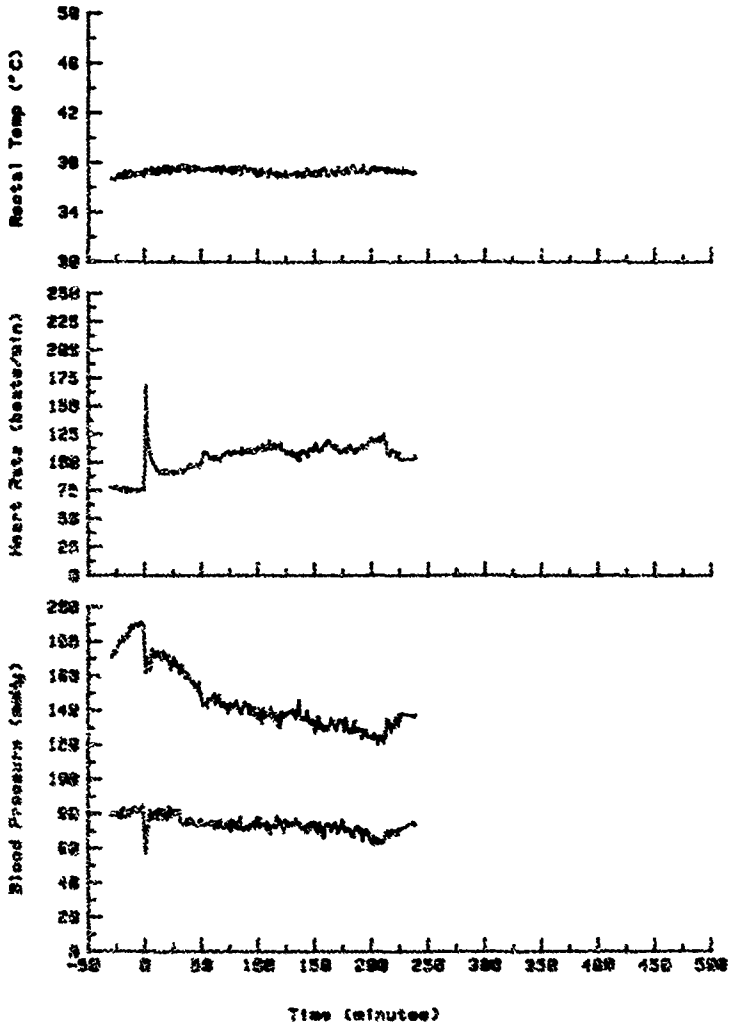


TABLE XXI.
EXPERIMENTAL DATA FOR DOG 474
Body Weight: 13.38 kg
PGDN Dose - 4 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	53	96	7.18				3.21
-30	43	100	7.27				1.81
-15	40	102	7.30			17.5	
0						4.5	
5				2988			
10				1654			
15				1454	282	2.1	
20				908			
25				782			
30	40	102	7.29	702	354	4.2	3.76
35				574			
40				520			
45				472	362	4.4	
50				427			
55				367			
60	41	103	7.29	350	253	7.6	3.55
70				276			
75					201	17.0	
80				235			
90	43	103	7.28	213	120	31.6	2.95
100				161			
105					117	40.0	
110				158			
120	39	106	7.29	149	84	33.0	2.41
130				181			
135					74	20.0	
140				111			
150	39	105	7.26	110	59	145.0	3.72
160				98			
165					51	193.0	
170							
180	39	106	7.28	91	32	79.5	3.28
190				78			
195					29	106.0	
200				78			

TABLE XXI. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	106	7.28	68		89.0	3.21
220				61			
225					19	44.0	
230				56			
240				52			2.82
255							
270							
285							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in urine

Figure 53. Rectal Temperature, Heart Rate and Blood Pressure of Dog A74

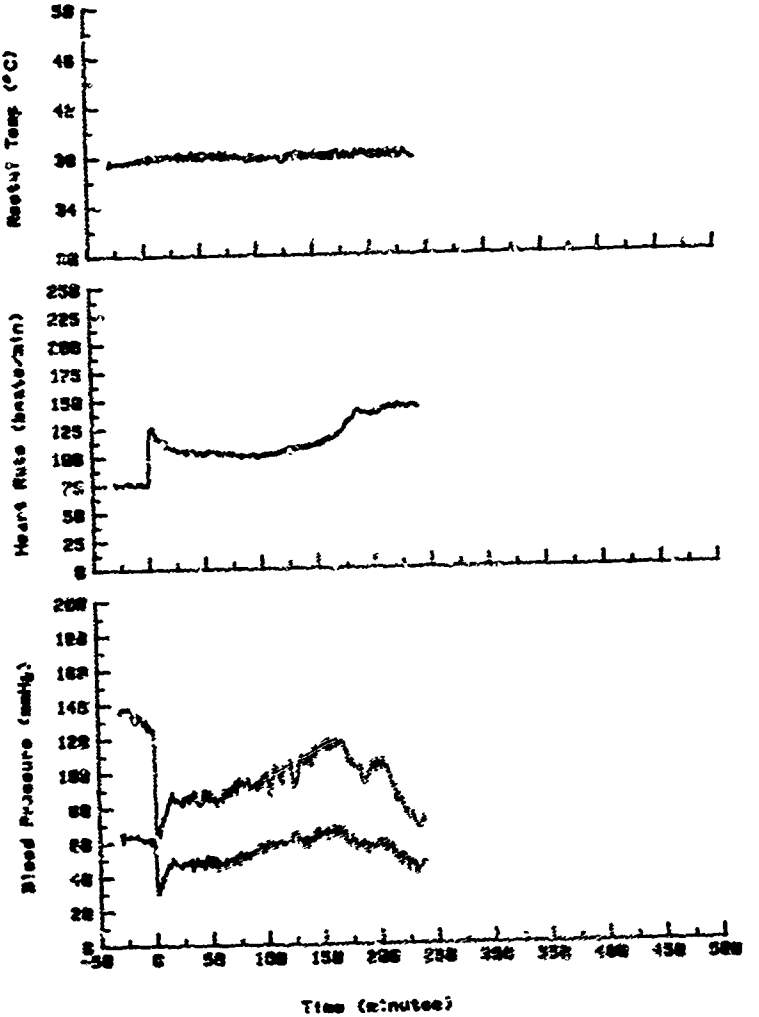


TABLE XXII.
EXPERIMENTAL DATA FOR DOG A90
Body Weight: 12.70 kg
PGDN Dose - 10 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	46	90	7.22				2.61
-30	38	99	7.29				2.05
-15	38	101	7.28				
0						3.8	
5				8832			
10				4201			
15				2887	322	13.5	
20				2419			
25				2076			
30	43	96	7.24	1768	1404	9.5	5.63
35				1467			
40				1362			
45				1185	1064	6.6	
50				1092			
55				1040			
60	41	99	7.27	1016	599	12.8	3.94
70				836			
75					542	18.0	
80				791			
90	42	100	7.25	647	409	36.5	4.52
100				698			
105					425	38.5	
110				645			
120	40	102	7.27	519	296	46.7	3.57
130				581			
135					271	65.0	
140				649			
150	41	101	7.26	389	182	70.0	6.60
160				391			
165					176	56.0	
170				350			
180	41	101	7.27	630	186	24.0	3.05
190				323			
195					145	20.0	
200				263			

TABLE XXII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	102	7.21	252	93	26.3	5.43
220				239			
225					99	20.8	
230				205			
240				205	84	23.3	2.83
255							
270							
285							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in Urine

Figure 54. Rectal Temperature, Heart Rate and Blood Pressure of Dog A90

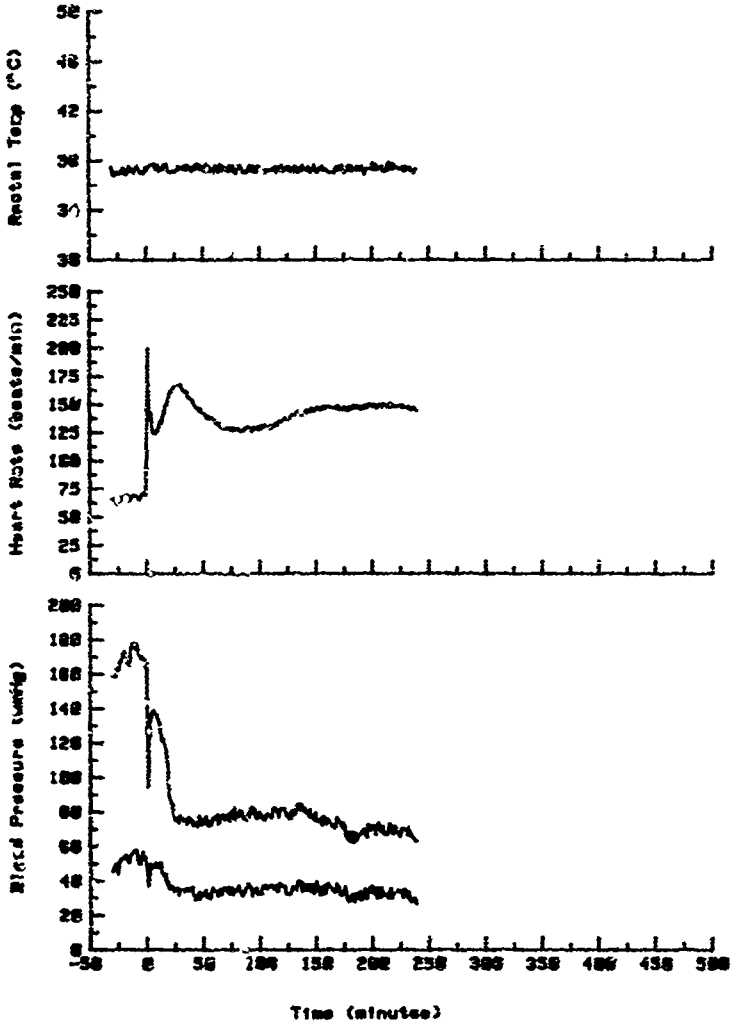


TABLE XXIII.
EXPERIMENTAL DATA FOR DOG A80
Body Weight: 12.36 kg
PGDN Dose - 10 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	40	109	7.33			16.2	2.90
-30	38	109	7.36			6.6	2.39
-15	40	104	7.34			14.0	
0						9.6	
5				7563			
10				4883			
15				3310	716	5.5	
20				2543			
25				2469			
30	46	92	7.28	2086	1723	11.0	6.19
35				1803			
40				1482			
45				1330	1411	5.8	
50				1315			
55				1167			
60	45	94	7.31	1079	1055	3.2*	5.39
70				901			
75					911	2.0*	
80				737			
90	44	95	7.33	691	686	3.5*	3.24
100				592			
105					499	1.5*	
110				527			
120	39	100	7.37	452	393	3.0*	2.78
130				428			
135					274	2.0*	
140				345			
150	39	98	7.39	310	248	2.0*	4.81
160				286			
165					209	1.8*	
170				252			
180	38	100	7.39	220	204	2.4*	2.79
190				208			
195					193	2.2*	
200				183			

TABLE XXIII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	39	96	7.37	179	115	1.6*	4.26
220				146			
225					85	2.2*	
230				146			
240	43	94	7.33	140	82	2.0*	3.37
255				111	62	1.8*	
270	44	93	7.32	99	72	1.6*	4.41
285				87		2.0*	
300	42	96	7.35	73	52	2.2*	3.01
315				55	32	1.6*	
330	40	98	7.37	50	30	3.0*	4.81
345				35	37	1.7*	
360	39	100	7.38	37	30	2.5*	2.41
375				33	22	1.8*	
390	38	104	7.36		23	2.0*	2.62
405					22	2.1*	
420	39	99	7.35			6.2	2.89
435						6.6	
450	39	99	7.35			4.2	3.45
465						16.6	
480						13.3	2.51

* Blood in urine

Figure 55. Rectal Temperature, Heart Rate and Blood Pressure of Dog A80

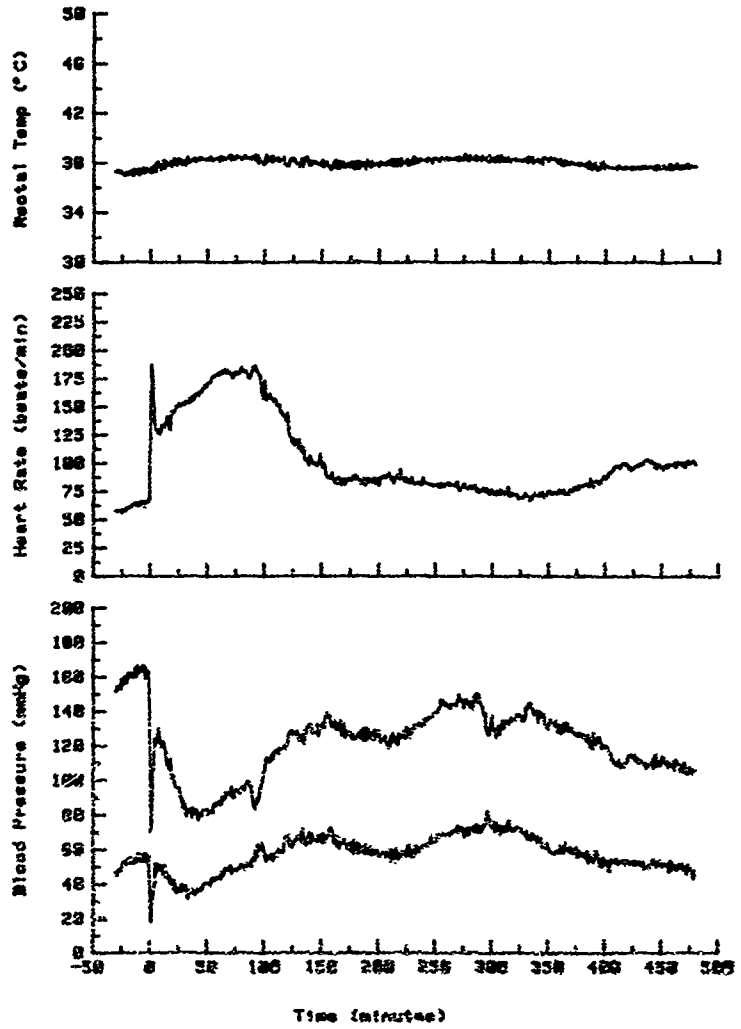


TABLE XXIV.
EXPERIMENTAL DATA FOR DOG A98
Body Weight: 10.89 kg
PGDN Dose: 10 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45							
-30	45	99	7.26			18.0	2.04
-15	38	105	7.33			5.4	
0							
5				6450			
10				4026			
15				3203	364	10.8	
20				2473			
25				2268			
30	41	98	7.30	1959	768	8.4	4.20
35				1682			
40				1400			
45				1364	1234	3.4	
50				1258			
55				1161			
60	38	99	7.31	1024	1004	16.4	3.52
70				944			
75					777	38.0	
80				756			
90	41	98	7.30	689	555	53.9	5.31
100				651			
105					418	54.0	
110				556			
120	38	99	7.31	600	377	82.0	3.71
130				504			
135					306	62.0	
140				518			
150	38	98	7.30	452	280	91.5	3.15
160				402			
165					272	81.0	
170				397			
180	41	97	7.28	381	251	103.5	3.61
190				358			
195					206	85.0	
200				294			

TABLE XXIV. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	42	98	7.29	286	142	84.0	2.25
220				265			
225					144	68.5	
230				244			
240	37	102	7.30	218	123	93.0	3.38
255				201	114	82.0	
270	37	100	7.29	188	85	77.0	2.68
285				168	72	55.0	
300	40	100	7.29	190	68	32.0	3.57
315				234	66	18.0	
330	39	100	7.29	126	53	5.0	4.94
345				113	54	19.5	
360	41	99	7.28	102	101	15.4	2.49
375				95	29	16.0	
390	38	98	7.27	81	23	31.0	1.97
405				61	23	36.5	
420	40	98	7.27	64	22	47.0	3.13
435				48	20	42.0	
450	41	97	7.27	43	17	31.2	2.99
465				43	15	24.5	
480				35	11	21.0	3.18

* Blood in urine

Figure 56. Rectal Temperature, Heart Rate and Blood Pressure of Dog A98

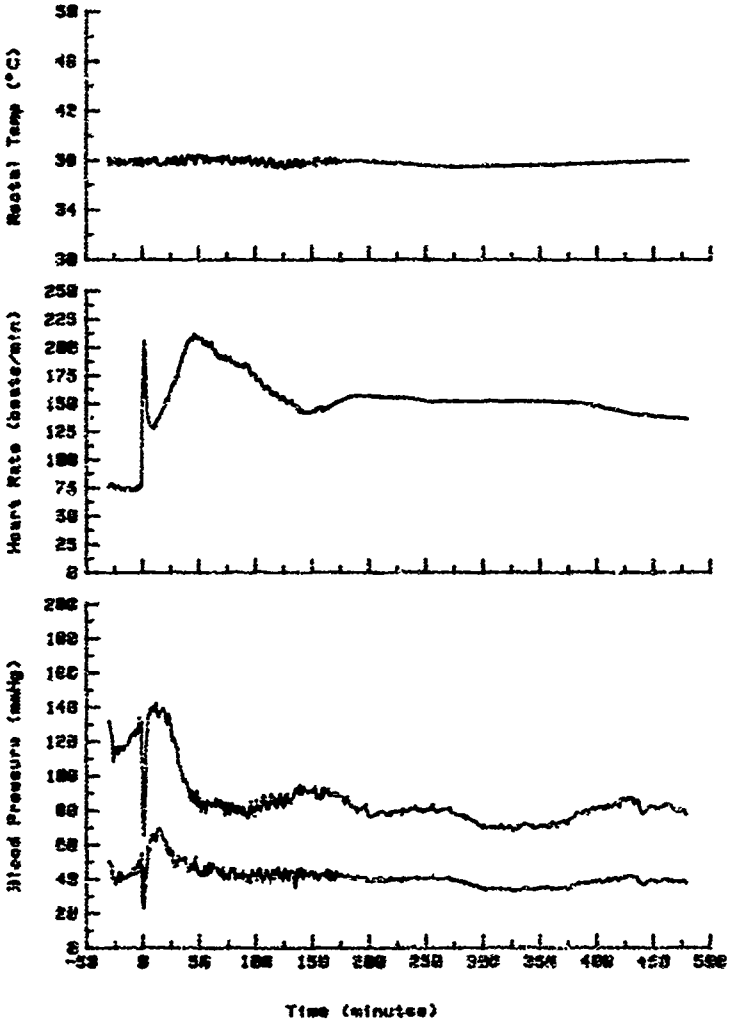


TABLE XXV.
EXPERIMENTAL DATA FOR DOG B20
Body Weight: 12.02 kg
PGDN Dose: 10 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	37	102	7.31			0.0	1.51
-30	37	96	7.31				2.34
-15	43	90	7.33				
0						11.5	
5				4903			
10				3056			
15	42	90	7.29	2631	3906	0.5	
20				2136			
25				1716			
30	42	92	7.31	1650	418	1.2	3.85
35				1358			
40				1305			
45				1206	751	3.3	
50				1106			
55				1022			
60	37	98	7.33	1054	631	10.4	2.73
70				907			
75						0.0	
80				818			
90	39	96	7.33	761	541	21.0	4.09
100				615			
105					380	23.4	
110				621			
120	38	94	7.31	582	315	48.0	5.55
130				523			
135					312	56.0	
140				494			
150	40	92	7.31	508	290	58.0	3.62
160				492			
165					285	76.5	
170				421			
180	40	92	7.31	468	247	79.0	5.51
190				430			
195					221	58.0	
200				403			

TABLE XXV. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	94	7.30	496	215	70.0	3.40
220				367			
225					191	54.0	
230				362			
240	40	94	7.30	394	208	65.0	2.86
255				342	190	98.0	
270	40	93	7.32	350	219	69.0	4.49
285				430	140	56.0	
300	39	93	7.33	321	143	66.5	2.23
315				310	144	51.0	
330	40	92	7.32	279	145	41.0	3.26
345				272	121	25.0	
360	41	92	7.32	274	118	16.0	3.13
375				254	113	8.0	
390	41	91	7.33	218	104	12.2	2.05
405				208	91	19.2	
420	42	89	7.32	219	96	33.0	2.51
435				184		23.0	
450	41	89	7.31	178	83	13.5	4.17
465				170	85	34.0	
480				169	88	78.8	3.54

* Blood in urine

Figure 57. Rectal Temperature, Heart Rate and Blood Pressure of Dog B20

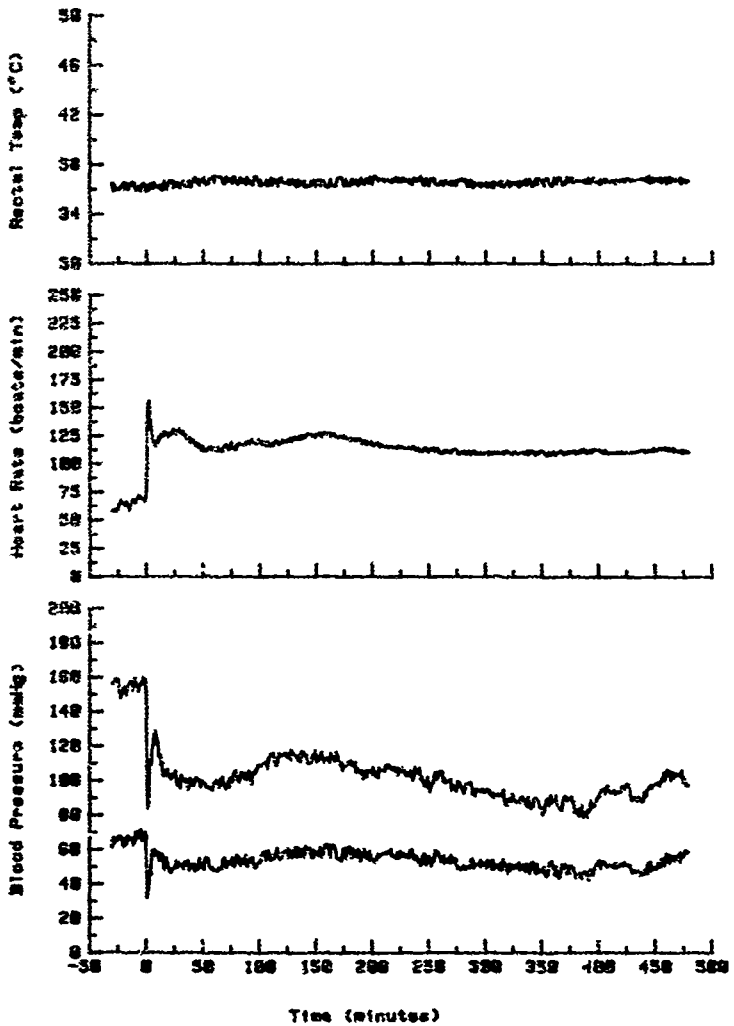


TABLE XXVI.
EXPERIMENTAL DATA FOR DOG 840
Body Weight: 9.75 kg
PGDN Dose: 10 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
							2.19
-45	37	106	7.34				
-30	43	95	7.31			0.0	2.28
-15	44	98	7.32			0.0	
0						36.0	
5				6914			
10				5053			
15				3449	779	8.5	
20				2611			
25				2163			
30	44	96	7.32	2164	1080	1.3	5.01
35				1823			
40				1706			
45				1691		0.0	
50				1296			
55				1239			
60	39	100	7.33	1180		0.0	4.07
70				958			
75					1287	8.0	
80				778			
90	42	100	7.32	1069	670	18.8	4.40
100				630			
105					498	28.0	
110				736			
120	40	101	7.34	600	443	26.0	3.28
130				425			
135					423	67.0	
140				422			
150	40	102	7.36		275	53.0	4.98
160				357			
165					237	85.0	
170				370			
180	36	103	7.39	308	195	53.5	3.96
190				280			
195					182	40.0	
200				262			

TABLE XXVI. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	37	101	7.39	232	151	27.0	3.40
220				200			
225					129	34.0	
230				200			
240	39	98	7.35		114	31.0	3.46
255				160	111	133.0	
270	42	96	7.33	158	97	81.0	5.17
285					80	49.5	
300	41	98	7.33	128	71	14.0	2.84
315				102	62	3.5	
330	40	100	7.34	98	46	4.0	3.57
345				91	35	5.3	
360	40	98	7.34	91	35	12.5	3.90
375					32	10.5	
390	40	98	7.32	74	31	3.5	5.60
405				62	28	7.0	
420	40	128	7.32	82	26	3.0	4.10
435					26	4.0	
450	44	126	7.32	47	25	2.6	4.67
455					19	2.0	
480				33	17	3.8	3.24

* Blood in urine

Figure 58. Rectal Temperature, Heart Rate and Blood Pressure of Dog B40

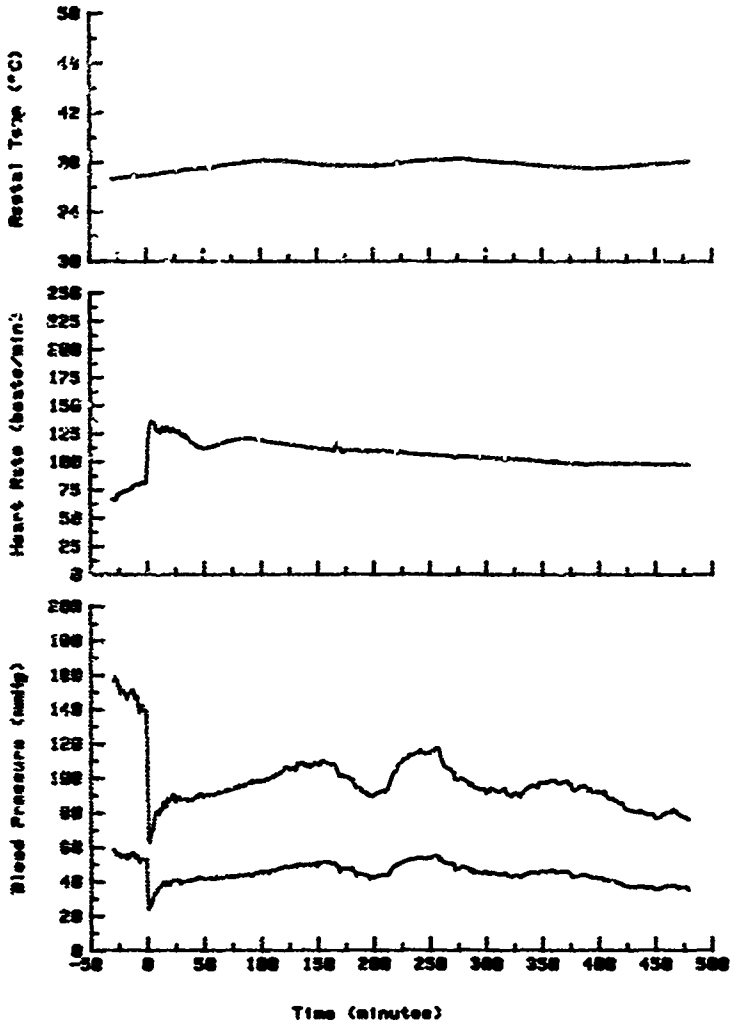


TABLE XXVII.
EXPERIMENTAL DATA FOR DOG A88
Body Weight: 12.02 kg
PGDN Dose: 40 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
							2.73
-45	40	112	7.29				2.53
-30							
-15	38	123	7.37			4.4	
0							
5				43649			
10				26107			
15				16281	3288	3.8	
20				12881			
25				10368			
30	40	93	7.25	9651	5909	3.0	5.18
35				7469			
40				6620			
45				6575	5770	2.0	
50				6540			
55				4891			
60	41	97	7.27	5026	4012	1.5	7.85
70				4377			
75					4154	1.6	
80				4112			
90	41	96	7.30	3956	3268	1.8*	9.14
100				3439			
105					2453	1.4*	
110				2913			
120	39	98	7.36	3120	2161	1.8*	8.46
130				2874			
135					2048	1.4*	
140				2575			
150	40	98	7.29	2422	1472	1.6*	9.19
160				2178			
165					1486	1.5	
170				1987			
180	38	99	7.30	2010	1321	1.6	6.00
190				1897			
195					1108	1.4	
200				1755			

TABLE XXVII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	36	101	7.31	1717	887	1.9	7.15
220				1557			
225					902	1.9	
230				1397			
240				1480	924	2.6	7.59
255							
270							
235							
300							
315							
330							
345							
360							
375							
390							
405							
420							
435							
450							
465							
480							

* Blood in urine

Figure 59. Rectal Temperature, Heart Rate and Blood Pressure of Dog A88

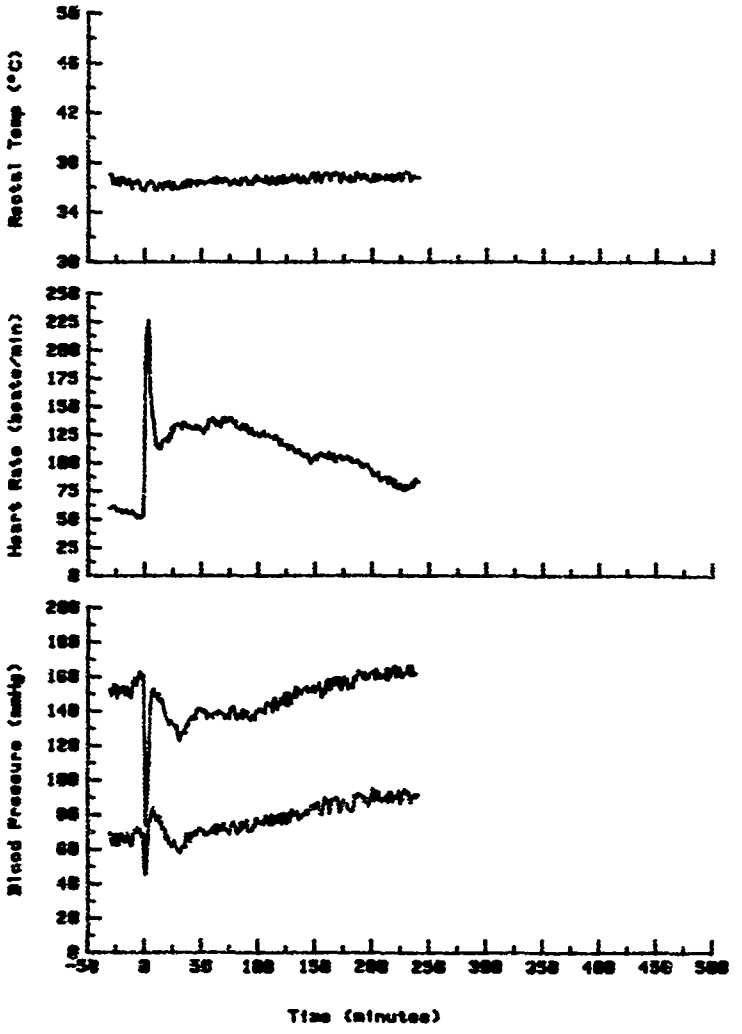


TABLE XXVIII.
EXPERIMENTAL DATA FOR DOG A76
Body Weight: 12.47 kg
PGDN Dose: 40 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	37	101	7.34				2.73
-30	41	96	7.36			37.0	2.15
-15	40	97	7.37			8.5	
0						4.2	
5				43449			
10				22874			
15				17254	5336	4.2	
20				13723			
25				10977			
30	43	92	7.34	9874	6794	1.2	5.75
35				8332			
40				7809			
45				6477		0.0	
50				5974			
55				5884			
60	40	95	7.35	5403	3152	1.6*	7.58
70				5024			
75						0.0	
80				4048			
90	40	94	7.36	3983	4193	5.4*	12.35
100				3396			
105					2003	20.8*	
110				3239			
120	41	94	7.36	3130	2164	55.4	10.95
130				3028			
135					2279	49.0	
140				2782			
150	42	92	7.34	2863	1677	40.0	9.83
160				2431			
165					1984	77.0	
170				2288			
180	39	95	7.35	2198	1541	59.0	6.51
190				2130			
195					1835	67.0	
200				2002			

TABLE XVIII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	95	7.37	2142	1426	68.0	11.08
220							
225					1384	62.5	
230				1868			
240	39	95	7.37	1813	1306	80.5	6.20
255				1737	1096	67.0	
270	39	94	7.36	1652	1126	37.0	8.24
285				1522	1148	60.5	
300	39	93	7.36	1430	871	121.0	7.90
315				1339	1001	80.0	
330	39	82	7.37	1179	812	53.0	7.86
345				1129	705	27.0	
360	39	94	7.38	1027	674	3.8	6.23
375				922	702	1.5	
390	41	93	7.35	938		0.0	7.03
405				904	909	3.0*	
420	42	94	7.36	797	492	3.4*	6.57
435				780	518	7.5*	
450	41	94	7.36	772	447	6.2*	8.05
465				689	479	5.4*	
480				696	909	2.4*	5.14

* Blood in urine

Figure 60. Rectal Temperature, Heart Rate and Blood Pressure of Dog A76

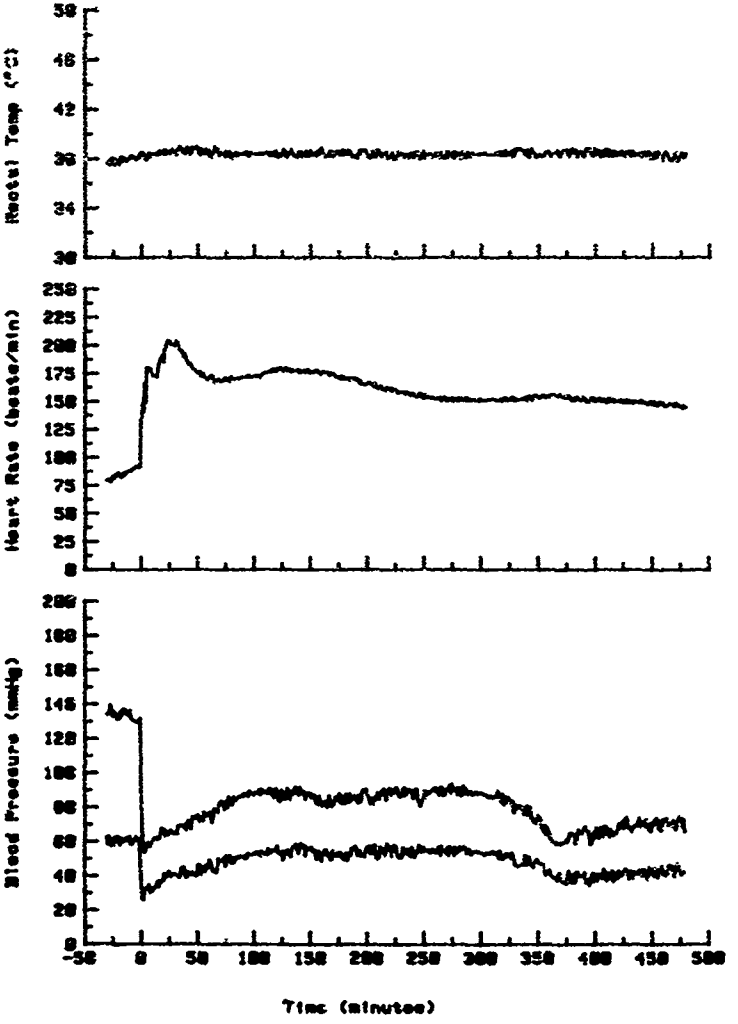


TABLE XXIX.
EXPERIMENTAL DATA FOR DOG A92
Body Weight: 10.89 kg
PGDN Dose: 40 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	39	104	7.31			23.0	2.74
-30	41	99	7.30			0.0	3.22
-15	43	99	7.31			3.2	
0						0.0	
5				35542			
10				20782			
15				18042	4836	4.2	
20				15442			
25				12552			
30	40	100	7.34	11325		0.0	8.08
35				9512			
40				8470			
45				8793		0.0	
50				8200			
55				7549			
60	38	97	7.36	7334		0.0	5.83
70				6630			
75						0.0	
80				5717			
90	44	92	7.33	5554	7653	6.4*	8.81
100				4701			
105					3860	1.8*	
110				4052			
120	40	96	7.35	4138		0.0	9.51
130				3725			
135						0.0	
140				3398			
150	41	95	7.33	2824	3649	5.0*	13.10
160				2344			
165						0.0	
170				2458			
180	42	95	7.32	2243	2654	2.8	10.49
190				2119			
195					1471	2.2*	
200				1950			

TABLE XXIX. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	98	7.34	1798	1250	2.4*	14.07
220				1578			
225					1079	2.0*	
230				1310			
240	43	97	7.33	1520	726	1.2*	7.83
255				1352		0.0	
270	39	96	7.34	1142	1578	1.6*	9.15
285				992	565	4.0*	
300	38	106	7.35	857	518	3.2*	7.96
315				774	481	2.0*	
330	40	104	7.35	640	389	2.5*	9.06
345				555	309	2.6*	
360	39	104	7.35	479	294	5.5*	6.59
375				416	237	5.2*	
390	39	107	7.35	373	217	8.3*	5.37
405				336	190	12.2*	
420	40	111	7.32	342	183	17.4	4.12
435				315	163	11.8	
450	44	109	7.29	286	183	56.4	4.83
465				246		60.5	
480				235		34.0	4.66

* Blood in urine

Figure 61. Rectal Temperature, Heart Rate and Blood Pressure of Dog A92

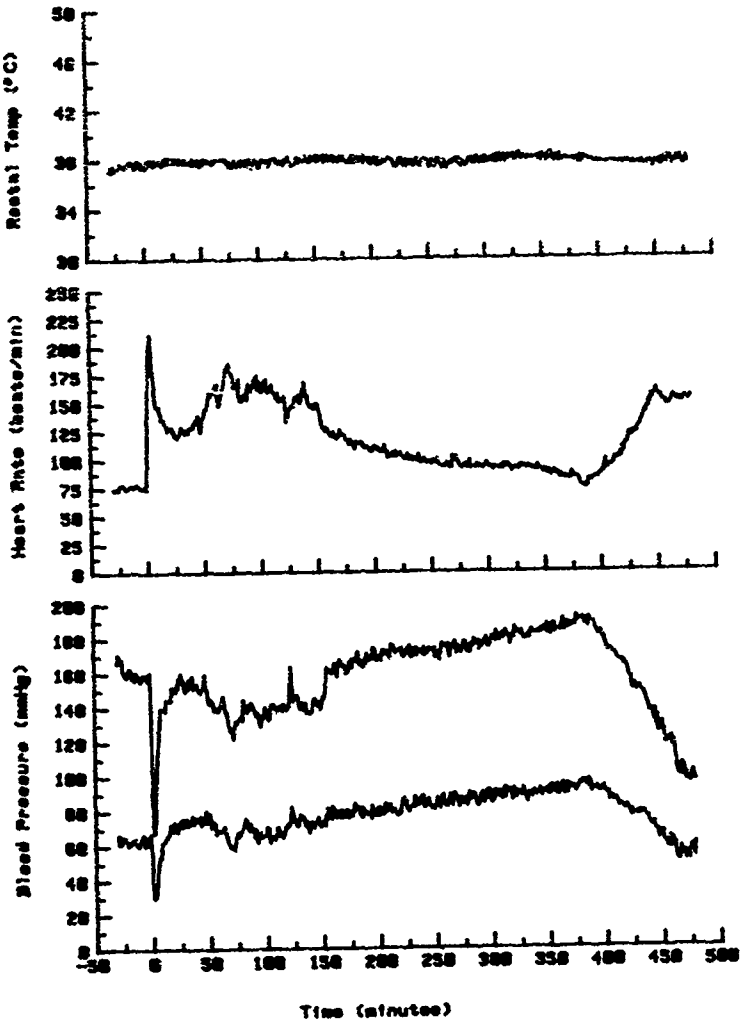


TABLE XXX.
EXPERIMENTAL DATA FOR DOG A94
Body Weight: 12.02 kg
PGDN Dose: 40 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	42	97	7.27			10.0	1.42
-30	38	99	7.33			2.5	2.07
-15	37	100	7.33			10.0	
0						3.0	
5				42087			
10				23482			
15				17760	1838	7.0	
20				14692			
25				12261			
30	40	91	7.30	10671	4853	1.4	5.70
35				9606			
40				8230			
45				7557		0.0	
50				6812			
55				6459			
60	40	90	7.29	6351	10643	1.0*	5.85
70				5554			
75					3557	3.2*	
80				4848			
90	42	90	7.29	4299	3683	6.5*	6.39
100				3898			
105					3085	12.4*	
110				3700			
120	42	92	7.29	3407	2496	18.6*	6.74
130				3244			
135					2460	25.8	
140				2899			
150	41	92	7.31	2997	2444	4.4	7.50
160				2599			
165					2079	8.0	
170				2518			
180	39	92	7.31	2480	1994	17.8	6.38
190				2195			
195					1875	6.0	
200				2195			

TABLE XXX. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	93	7.33	2156	1633	80.3	6.77
220				1985			
225					1456	27.3	
230				1854			
240	38	94	7.33	1829	1213	44.0	7.00
255				1742	1320	35.0	
270	40	93	7.30	1702	1184	33.0	5.97
285				1486	1313	25.0	
300	41	93	7.30	1416	972	35.5	4.76
315				1422	965	10.0	
330	43	93	7.29	1285	969	9.6*	8.57
345				1291	852	2.3*	
360	42	93	7.28	1335	772	3.8*	6.23
375				1138	644	4.8*	
390	44	95	7.25	1716	646	21.6	6.20
405				1019	655	36.0	
420	40	96	7.29	1052	605	122.5	6.02
435				915	599	117.0	
450	38	97	7.31	902	520	87.0	6.11
465				811	505	35.0	
480				736	507	5.7	6.55

* Blood in urine

Figure 62. Rectal Temperature, Heart Rate and Blood Pressure of Dog A94

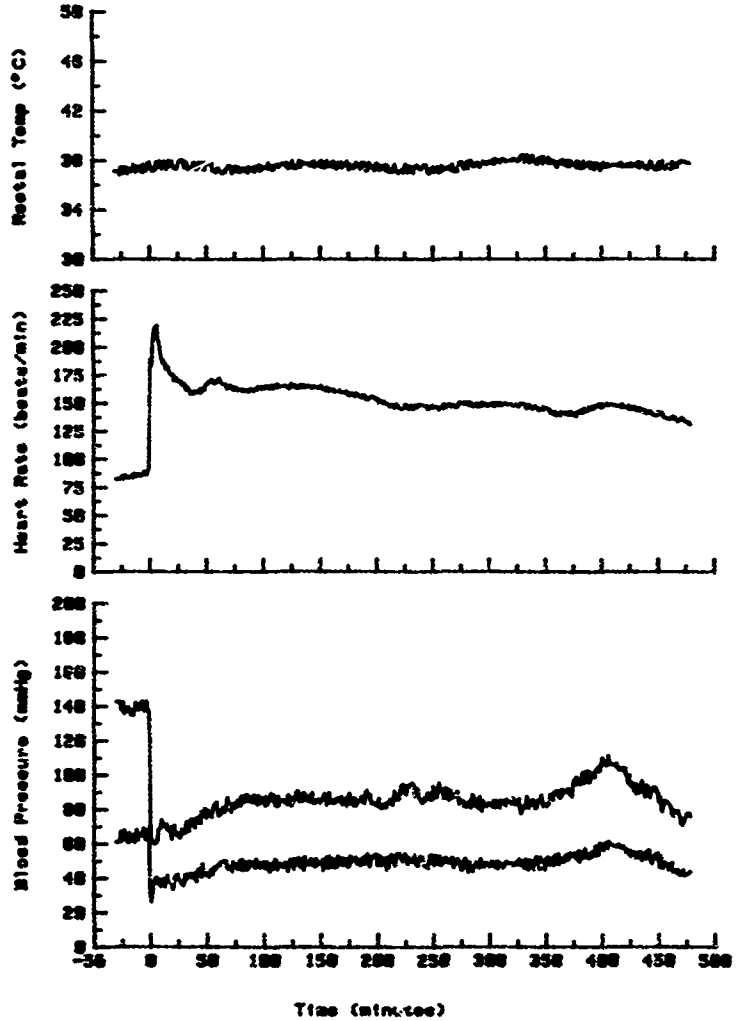


TABLE XXXI.
EXPERIMENTAL DATA FOR DOG B08
Body Weight: 10.32 kg
PGDN Dose: 40 mg/kg

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	36	110	7.34			5.5	2.38
-30	34	103	7.33			7.5	2.28
-15	42	94	7.29			2.0	
0						1.5	
5				35196			
10				22978			
15	40	97	7.29	17025		0.0	
20				13171			
25				11827			
30	40	96	7.28	11332		0.0	7.46
35				9362			
40				9033			
45				7958		0.0	
50				7259			
55				7098			
60	41	95	7.27	8527		0.0	7.66
70				6140			
75						0.0	
80				5398			
90	40	96	7.27	4910		0.0	9.81
100				4832			
105					14646	1.2*	
110				4462			
120	39	97	7.27	4158	2947	2.2*	7.50
130				3921			
135					3394	2.5*	
140				3838			
150	38	97	7.27	3664	2784	5.0*	9.74
160				3398			
165					2736	4.0*	
170				3100			
180	41	94	7.26	3185	2516	4.6*	9.51
190				3287			
195					2090	8.2*	
200				3105			

TABLE XXXI. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	97	7.28	2959	2000	25.6*	14.49
220				2645			
225					1686	23.0	
230				2514			
240	40	94	7.29	2555	1595	33.0	8.62
255				2388	1685	35.0	
270	40	96	7.31	2485	1532	37.0	9.92
285				2058	1453	29.0	
300	40	96	7.31	2022	1313	26.0	7.73
315				1842	1552	19.0	
330	39	97	7.32	1742	1278	10.8	7.82
345				1602	1187	6.4*	
360	40	96	7.30	1507	1251	5.0*	6.76
375				1395	973	11.0*	
390	43	96	7.30	1257	880	5.8*	7.61
405				1256	824	31.0	
420	39	99	7.30	1103	652	37.0	3.19
435				1172	616	35.0	
450	40	99	7.30	1091	542	24.0	4.93
465				1040	556	13.3	
480				963	446	15.3	4.52

* Blood in urine

Figure 63. Rectal Temperature, Heart Rate and Blood Pressure of Dog B08

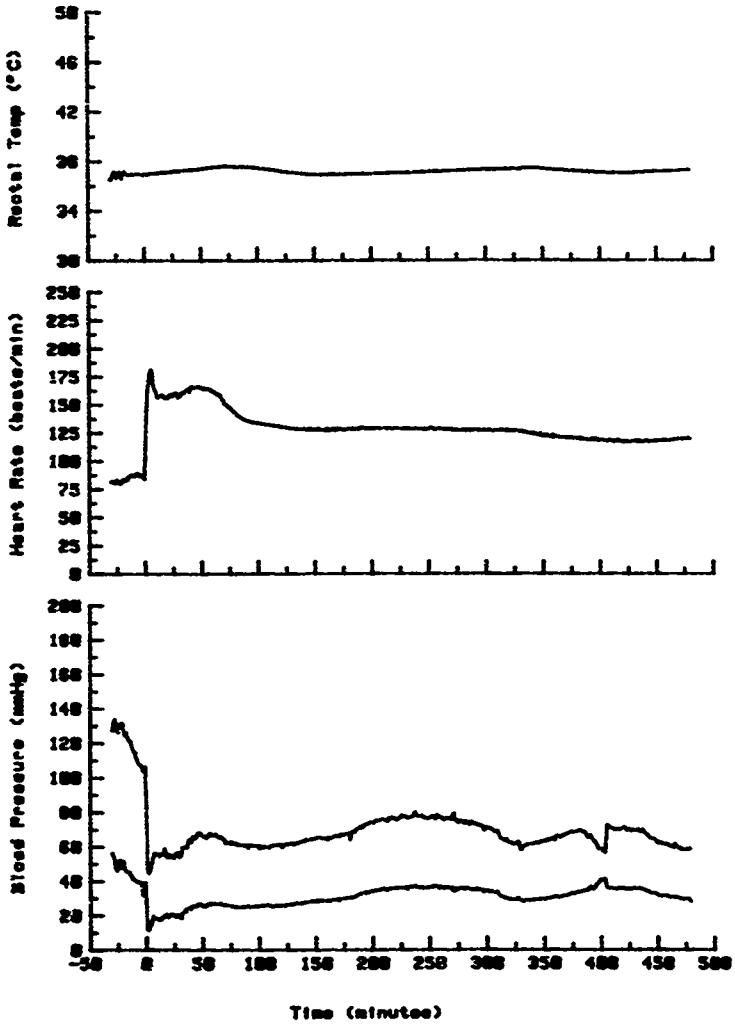


TABLE XXXII.
EXPERIMENTAL DATA FOR DOG A86
Body Weight: 12.13 kg
PGDN Dose: 100 mg/kg

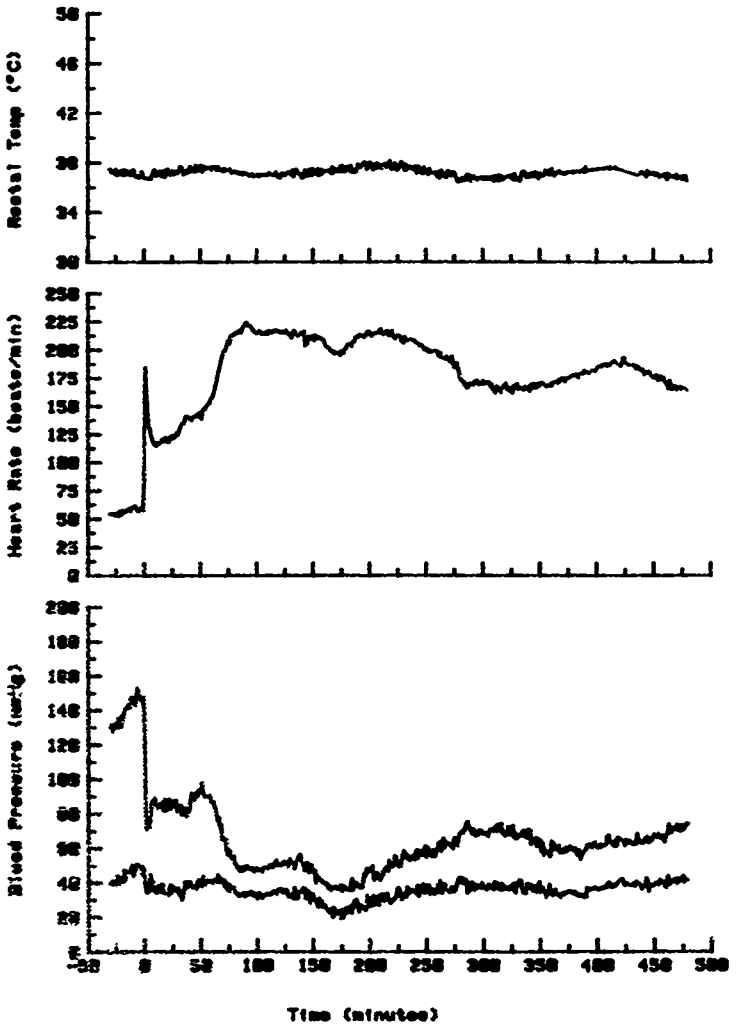
Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
-45	40	106	7.24				
-30						12.0	3.99
-15	38	110	7.30				3.26
0							
5						3.2	
10				110451			
15				78158			
20				61597	13499	1.3	
25				54519			
30	46	94	7.22	50870			
35				44575	18938	1.2*	20.78
40				39356			
45				38296			
50				35112		0.0	
55				31870			
60	44	97	7.28	29754			
70				26526	37628	1.2	29.86
75				25861			
80					19632	0.4	
90	41	93	7.31	22433			
100				20907		0.0	38.90
105				17649			
110					83441	0.2	
120	37	91	7.29	16272			
130				16215	9954	0.6	40.10
135				14677			
140						0.0	
150	43	87	7.30	14720			
160				13708		0.0	41.86
165				12292			
170					16310	1.6	
180	37	88	7.32	11139			
190				10666	3999	1.0	42.75
195				10696			
200					10709	0.6	
				8676			

TABLE XXXII. (CONTINUED)

Time (min)	P_aCO_2 (mmHg)	P_aO_2 (mmHg)	pH	[PGDN] blood (ng/ml)	[PGDN] urine (ng/ml)	Urine Vol (ml)	MetHb (%)
210	40	89	7.28	7770	6393	2.8	48.01
220				7573			
225					5272	1.4	
230				6972			
240	43	87	7.27	6460	4683	4.4	41.19
255				6210	4902	4.3	
270	40	89	7.29	5323	4175	1.4	42.71
285				4687	4034	1.5	
300	42	91	7.29	4299	3816	1.7	37.18
315				4207	4188	1.0	
330	40	89	7.27	3481	3175	5.7	40.37
345				3362	2681	3.0	
360	41	90	7.28	2938	1894	1.0	35.62
375				2670	2798	3.4	
390	40	88	7.30	2508	1666	2.3	34.87
405				2089	1726	4.4	
420	41	88	7.30	1942	1947	1.4	30.28
435				1850	1668	3.7	
450	38	92	7.30	1554	1066	3.5	31.55
465				1375	1067	2.0	
480	37	92	7.30	1285	958	2.4	25.38

* Blood in urine

Figure 64. Rectal Temperature, Heart Rate and Blood Pressure of Dog A86



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